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**OFFICE OF THE SCIENTIFIC DIRECTOR  
DIVISION OF INTRAMURAL RESEARCH, NIAID**

**1995 Annual Report  
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00020-20

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies with POLYICLC

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Hilton B. Levy, Ph.D., Special Assistant to the Scientific Director, NIAID

COOPERATING UNITS (if any)

Dr. Andre Salazar, Walter Reed; Dr.Meir Kende, USAMRIID; Dr. Radha Maheshwari, USUHS; Dr. Jonathan Wong, Canadian Defense Dept.

LAB/BRANCH

Office of the Scientific Director (OSD)

SECTION

INSTITUTE AND LOCATION

NIH/NIAID/DIR Building 14B South, Rm 300, Bethesda, MD 20892

TOTAL STAFF YEARS:

.50

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects   ☒ (b) Human tissues   ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

PolyICLC continues to be used in the treatment of anaplastic astrocytoma at Walter Reed. Sixteen patients have been on the drug for a mean of 46 months. All are in good health and carry on full normal lives. The expected 50% survival time for these patients would have been 24 months.

In collaboration with Dr. Wong of the Canadian defense ministry we have microencapsulated polyICLC into multilammellar liposomes. As noted below, this prolongs the duration of action of the drug. Together with Dr.Maheshwari of USUHS and the Central Drug Research Institute of Lucknow, India, we have shown that polyICLC has a strong cytotoxic action vs. Leishmania donovani in hamsters. Thirty one multiple sclerosis patients have been on polyICLC during the past ten years. Most of the 31 patients have shown a strong decrease in the rate of progression of the disease, some have stabilized, and some have improved.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 AI 00662-04 ODIR						
<b>PERIOD COVERED</b> October 1, 1994 to September 30, 1995								
<b>TITLE OF PROJECT</b> <i>Characterization of the FcεRI α chain by crystallography</i>								
<b>PRINCIPAL INVESTIGATOR</b> PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID Others: <table style="width: 100%; margin-top: 5px;"> <tr> <td style="width: 33%;">Jami W. Brown</td> <td style="width: 33%;">Microbiologist</td> <td style="width: 33%;">MAIS, NIAID</td> </tr> <tr> <td>Salvatore Sechi</td> <td>Visiting Associate</td> <td>MAIS, NIAID</td> </tr> </table>			Jami W. Brown	Microbiologist	MAIS, NIAID	Salvatore Sechi	Visiting Associate	MAIS, NIAID
Jami W. Brown	Microbiologist	MAIS, NIAID						
Salvatore Sechi	Visiting Associate	MAIS, NIAID						
<b>COOPERATING UNITS</b>  								
<b>LAB/BRANCH</b> Office of Scientific Director (OSD)								
<b>SECTION</b> Molecular Allergy and Immunology Section								
<b>INSTITUTE AND LOCATION</b> NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852								
<b>TOTAL STAFF YEARS:</b> 2.2	<b>PROFESSIONAL:</b> 2.2	<b>OTHER:</b>  						
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews								
<b>SUMMARY OF WORK</b> <p>One of the critical steps in allergic reactions is the binding of IgE to its high affinity receptor (FcεRI). The extracellular domain of the α-chain (α-t) of the FcεRI is solely responsible for the binding to IgE, and the Fc portion of IgE is sufficient for binding to FcεRI. For a complete understanding of the interaction between FcεRI and IgE the structure of the two proteins and of their complex has to be determined. This project is aimed to obtain the structure of α-t and of its complex with Fc. Knowledge of the α-t structure or of its complex with Fc would allow the design of novel therapeutic agents. We have expressed α-t capable of binding IgE in CHO cells. However, glycosylation heterogeneity prevented crystallization. On the other hand the deglycosylated α-t produced in <i>E.Coli</i> was unstable and had tendency to aggregate. For this reason we have produced a preparation of α-t with limited glycosylation. These proteins lead to crystal that could diffract to a resolution of 3.5Å. A data set 83% complete at 4 Å resolution was collected using a synchrotron as an X-ray source. Screening for heavy atoms derivatives and molecular replacement studies are in progress. He have expressed the Fc as a soluble protein and in inclusion body in <i>E.coli</i>. We hope to be able to produce milligrams amounts of this Fc for making cocrystal with α-t. In addition we have found that a major conformational rearrangements occurs on the molecule of IgE after binding to α-t.</p>								



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 AI 00664-03 ODIR												
PERIOD COVERED October 1, 1994 to September 30, 1995														
TITLE OF PROJECT <i>Distribution and role of FcεRI in human cells</i>														
PRINCIPAL INVESTIGATOR  PI:           Jean-Pierre Kinet, Section Chief   MAIS, NIAID  Others: <table style="width: 100%; margin-top: 10px;"> <tr> <td style="width: 33%;">K. Ochiai</td> <td style="width: 33%;">Visiting Fellow</td> <td style="width: 33%;">MAIS, NIAID</td> </tr> <tr> <td>M-H. Jouvin</td> <td>Visiting Associate</td> <td>MAIS, NIAID</td> </tr> <tr> <td>D. Dombrowicz</td> <td>Visiting Fellow</td> <td>MAIS, NIAID</td> </tr> <tr> <td>V. Flamand</td> <td>Special Volunteer</td> <td>MAIS, NIAID</td> </tr> </table>			K. Ochiai	Visiting Fellow	MAIS, NIAID	M-H. Jouvin	Visiting Associate	MAIS, NIAID	D. Dombrowicz	Visiting Fellow	MAIS, NIAID	V. Flamand	Special Volunteer	MAIS, NIAID
K. Ochiai	Visiting Fellow	MAIS, NIAID												
M-H. Jouvin	Visiting Associate	MAIS, NIAID												
D. Dombrowicz	Visiting Fellow	MAIS, NIAID												
V. Flamand	Special Volunteer	MAIS, NIAID												
COOPERATING UNITS  -G. Stingl (Vienna, Austria) -M. Capron (Lille, France) and A. Brini (Milano, Italy)														
LAB/BRANCH Office of Scientific Director (OSD)														
SECTION Molecular Allergy and Immunology Section														
INSTITUTE AND LOCATION NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852														
TOTAL STAFF YEARS: 2.2	PROFESSIONAL: 2.2	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK  This project was terminated.														





## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00665-04 ODIR

## PERIOD COVERED

October 1, 1994 to September 30, 1995

## TITLE OF PROJECT

*The role of the FcεRI receptor subunits and of the receptor associated molecules in signal transduction*

## PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

## Others:

S. Lin	IRTA Fellow	MAIS, NIAID
M. H. Jouvin	Visiting Scientist	MAIS, NIAID
R. Paolini	Special Volunteer	MAIS, NIAID
A. Scharenberg	Special Volunteer	MAIS, NIAID

## COOPERATING UNITS

NONE

## LAB/BRANCH

Office of Scientific Director (OSD)

## SECTION

Molecular Allergy and Immunology Section

## INSTITUTE AND LOCATION

NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

## TOTAL STAFF YEARS:

4.0

## PROFESSIONAL:

4.0

## OTHER:

0.0

## CHECK APPROPRIATE BOX(S)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK

The high affinity IgE receptor (FcεRI) belongs to a class of receptor which lacks intrinsic enzymatic activity but activates non-receptor tyrosine and serine/threonine kinases and phosphatases. This activation leads to the phosphorylation and activation of phosphatidylinositol-specific phospholipase C, an increase in intracellular calcium and ultimately to cell degranulation and the release of the mediators of allergic reactions. We have previously established that the β chain of FcεRI binds the tyrosine lyn and that the γ chain of FcεRI activated the tyrosine kinase syk. Using various recombinant vaccinia viruses engineered in the laboratory, we have no reconstituted, in a null fibroblastic cell line, a minimal signaling complex that includes the αβγ<sub>2</sub> FcεRI and the tyrosine kinases lyn and syk. In this complex like in a mast cell spontaneously expressing FcεRI, clustering induces receptor tyrosine phosphorylation and syk activation. Upon triggering, lyn phosphorylates the β and γ chains and phosphorylates and activates syk. These events do not require a hematopoietic-specific phosphatase. However, an unidentified phosphatase present in this fibroblastic cell line is probably responsible for preventing triggering-independent phosphorylation of the receptor. Triggering through this minimal complex does not result in the phosphorylation of substrates downstream of syk similar to the ones that are phosphorylated after triggering of a mast cell. We are currently exploring the possibility that other elements are part of the signaling complex of FcεRI and could be responsible for the involvement of downstream elements resulting in degranulation and the release of allergy mediators.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 AI 00668-04 ODIR									
PERIOD COVERED October 1, 1994 to September 30, 1995											
TITLE OF PROJECT <i>Production of IgE receptor deficient mice by homologous recombination</i>											
PRINCIPAL INVESTIGATOR  PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID Others: <table style="width: 100%; margin-top: 5px;"> <tr> <td style="width: 33%;">V. Flamand</td> <td style="width: 33%;">Visiting Fellow</td> <td style="width: 33%;">MAIS, NIAID</td> </tr> <tr> <td>D. Dombrowicz</td> <td>Visiting Fellow</td> <td>MAIS, NIAID</td> </tr> <tr> <td>M-H. Jouvin</td> <td>Visiting Scientist</td> <td>MAIS, NIAID</td> </tr> </table>			V. Flamand	Visiting Fellow	MAIS, NIAID	D. Dombrowicz	Visiting Fellow	MAIS, NIAID	M-H. Jouvin	Visiting Scientist	MAIS, NIAID
V. Flamand	Visiting Fellow	MAIS, NIAID									
D. Dombrowicz	Visiting Fellow	MAIS, NIAID									
M-H. Jouvin	Visiting Scientist	MAIS, NIAID									
COOPERATING UNITS  Beverly Köller (The University of North Carolina) and Alan Sher (NIAID) Joseph Urban (USDA, Beltsville, Maryland) and Stephen Galli (Beth Israel Hospital, Boston)											
LAB/BRANCH Office of Scientific Director (OSD)											
SECTION Molecular Allergy and Immunology Section											
INSTITUTE AND LOCATION NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852											
TOTAL STAFF YEARS: 3.2	PROFESSIONAL: 3.2	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK  <p>             Mast cells and basophils, which are activated by immunoglobulin E (IgE) and allergen, play a prominent role in anaphylaxis. However, they express at least three types of IgE receptors: the high affinity IgE receptor (FcεRI), and the two low affinity IgG receptors (FcγRII and FcγRIII) also capable of binding IgE. We have generated mice deficient with a disrupted FcεRI α chain gene. This defect results in complete suppression of the cell surface expression of FcεRI. These mice appear normal and express a normal number of mast cells, but they are resistant to cutaneous and systemic anaphylaxis. These data demonstrate that FcεRI is necessary for the initiation of IgE-dependent anaphylactic reactions. We are now analyzing the response of our FcεRI deficient mice in active anaphylaxis experiments. In humans, IgE and FcεRI present on eosinophils have also been shown to play a prominent role in the defense against parasites. In experimental infections with <i>Schistosoma mansoni</i>, <i>Nippostrongylus brasiliensis</i> and <i>Heligmosomoides polygyrus</i>, no difference was observed between the FcεRI deficient mice and normal animals suggesting that in mice, anti-helminthic immunity was not mediated through FcεRI. We have generated mice with a disrupted FcεRI β chain gene. Those animals are also resistant to anaphylaxis. In humans, conflicting data have been correlating atopy with mutations in the β gene and its maternal imprinting. We have used mice carrying one copy of the disrupted allele inherited from either parents to show that, at least in rodents, there was no imprinting of the β gene. The β chain also associates with FcγRIII and we are investigating its contribution in the FcγRIII-mediated cell activation. We have also obtained mice with both α and β disrupted.           </p>											





Laboratory of Cellular and Molecular Immunology

1995 Annual Report  
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00486-09	T Cell Differentiation - Fowlkes	7 - 11
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PHS-NIH  
Summary Statement  
Office of the Chief  
Laboratory of Cellular and Molecular Immunology  
October 1, 1994 through September 30, 1995

## Introduction

The Laboratory of Cellular and Molecular Immunology was created in November, 1986. Its major task is to perform research on thymus-derived (T) lymphocytes. Its objective is to understand how these critical basic cells of the immune system differentiate and function. The approach is to define problems in whole animal systems, set up *in vitro* analogs in tissue culture, and determine the molecular basis for the phenomenon. The Laboratory employs techniques from the disciplines of cellular immunology, molecular biology, and protein biochemistry.

During the 9th year, the Laboratory has pursued seven major areas of research: thymic stem cells, development of  $\gamma\delta$  T cells, positive selection of  $\alpha\beta$  T cells, isolation of genes involved in thymic function, molecular aspects of T cell costimulation and anergy, molecular mechanisms of programmed cell death, and a new model to explain tolerance and responsiveness in the immune system.

## T Cell Activation

Over the past several years, we have been studying two phenomena in cloned populations of CD4<sup>+</sup> T lymphocytes referred to as costimulation and anergy. The former entails a 30-100 fold enhancement of interleukin-2 (IL-2) production when signaling through the antigen-specific T cell receptor is supplemented with signaling through the CD28 receptor on the same cell. Anergy is an anti-proliferative state that the T cell enters if it only receives a signal through the antigen-specific receptor. Our recent work has focused on the molecular mechanisms behind these two phenomena. Using IL-2 RNA accumulation and transcription reporter assays, CD28 costimulation was shown to increase the stability of IL-2 messenger(m) RNA and not to enhance the initiation of transcription. An early component of the CD28 effect was nuclear, however, as the enhancement was found in unspliced RNA and for reporter constructs containing the 3' end of the IL-2 gene. The hypothesis we are currently testing is that CD28 costimulation causes the binding or increased stability of RNA shuttle proteins that attach to IL-2 RNAs in the nucleus and accompany them out into the cytoplasm, stabilizing the RNA from degradation in both compartments.

Our previous work on anergy provided evidence that the transactivation of the IL-2 gene by the AP-1 transcription factor was impaired in anergized T cell clones. We have now extended those findings with a variety of new reporter constructs to show that both the distal and proximal AP-1 response elements in the IL-2 enhancer are necessary for anergy induction. Anergy does not affect the induction of new Fos and Jun proteins which make up the AP-1 complex and has only a minor effect on the amount of complex which binds (2-fold less) and





its relative affinity for the AP-1 consensus sequence (<2-fold difference). We are currently testing the hypothesis that anergy blocks the induction of AP-1 transactivating activity.

[S. Kitagawa, J. Salo, J. Ragheb, D. Pham, L. Chiodetti, and R. H. Schwartz]

## **Thymus Subsets**

In order to understand the development and functioning of the thymus, both in terms of T cell differentiation and stromal cell environmental support, we have undertaken a molecular approach to identify genes that are uniquely expressed in this organ. Several strategies are currently being explored to accomplish this goal. One is to make cDNA libraries from fetal thymic organ cultures treated with deoxyguanosine and anti-CD45 to remove T cells and other hematopoietic cells. Subtractive hybridization approaches are then undertaken to further enrich for stromal cell cDNAs specific to the organ. The remaining cDNAs are then sequenced to identify new genes. Novel cDNAs are in turn tested by Northern Blot analysis and *in situ* hybridization to check for selective expression in thymic stromal cells. Although this project is still in its early phases, we have already sequenced 288 cDNAs and identified 182 with novel sequences. Two of these appear to be expressed in a thymic cortical epithelial cell line and not in a thymic medullary epithelial cell line. The full sequences of these two cDNAs are now being determined and detection of their expression in thymic sections by *in situ* hybridization is underway.

[M. Kim, Y. Zou, S. Wells, F. Flomerfelt, and R.H. Schwartz]

## **Molecular Mechanisms of Programmed Cell Death**

T cells recognize peptides bound to MHC molecules using a clonotypic receptor (TCR). Depending on the differentiation state of the T lymphocyte (i.e., responsiveness to a second signal) and/or the context in which the antigenic peptide is presented (i.e., availability of a second signal), this interaction can initiate distinct molecular programs, leading to opposite outcomes (T cell activation, unresponsiveness or death). A precise regulation of these responses results in a system which is both immunocompetent (able to be activated by and eliminate dangerous microorganisms) and self tolerant (able to eliminate or anergize T cell populations bearing TCRs specific for self antigens). The main process through which immunotolerance is achieved involves elimination by programmed cell death (apoptosis) of potentially dangerous T cells (negative selection or clonal deletion). Our main objective is the identification of the genes involved in negative selection and the characterization of the biochemical processes responsible for their functional activation. To this end, we have used a mouse T-cell hybridoma (3DO) as an *in vitro* model. Briefly, we have constructed cDNA libraries in eukaryotic expression vectors using an mRNA source enriched in apoptotic genes. Upon transfection of the libraries, 3DO has been triggered for apoptosis and recombinant plasmids have been recovered from surviving cells. This sub-library should be enriched in cDNAs able to block death by: 1) expressing in the cells adequate levels of specific antisense RNAs or dominant negative mutants for "apoptotic" genes;



and 2) producing "anti-apoptotic" proteins. At the present time, we have identified six novel genes whose overexpression in 3DO inhibits death. Northern Blot analysis indicates that they are not ubiquitous and have distinct patterns of tissue distribution. Of interest, four of them are either induced or repressed during T-cells apoptosis both *in vitro* and *in vivo*. For three of them we have cloned the complete coding sequence and expressed the recombinant protein in bacteria to produce specific antisera. Once this reagent will be available, the requirements for the functional activation of these molecules will be studied in more detail. In addition, we have isolated genomic clones for two of these genes. They will be used to identify the sequences regulating the transcription of these genes and to generate mice lacking their expression. Once obtained, the mutant mouse will be used as an *in vivo* animal model to study the physiological functions of the genes we have identified.

[P. Vito, E. Lacana, and L. D'Adamio]

## T Cell Differentiation

During development productive germline rearrangement must take place in both of the TCR loci,  $\alpha$  and  $\beta$ , or  $\gamma$  and  $\delta$  before a TCR heterodimer can be expressed. It has been proposed that commitment to the  $\alpha\beta$  or  $\gamma\delta$  lineages takes place prior to or independent of the rearrangement process. TCR class exclusion could then occur by targeted gene rearrangement or by blocking rearrangements, transcription, or translation of the other TCR loci. Alternatively, the productive rearrangement and/or expression of a particular TCR heterodimer,  $\alpha\beta$  or  $\gamma\delta$ , could determine the lineage choice. In many TCR $\alpha\beta$  transgenic mice, we find that no lymphoid  $\gamma\delta$  T cells develop but instead an unusual population of CD4<sup>+</sup>8<sup>+</sup> cells appear, bearing the  $\alpha\beta$  transgenic receptor, but with phenotypic and functional properties of  $\gamma\delta$  T cells. The results suggest that commitment to the  $\alpha\beta$  versus  $\gamma\delta$  lineage is independent of TCR rearrangement and of TCR expression or usage. To better understand how TCR class exclusion operates, we have used a highly quantitative PCR-ELISA-assay to show that TCR $\gamma$  V to J rearrangements occur in the major  $\alpha\beta$  lineage of both transgenic and nontransgenic mice as well as in the unusual  $\alpha\beta^+$  CD4<sup>+</sup>8<sup>+</sup> cell population of TCR $\alpha\beta$  transgenic mice. Thus, the presence and expression of endogenous or transgenic TCR $\alpha\beta$  does not prevent rearrangement at the  $\gamma$  or  $\delta$  loci. Moreover, in other  $\alpha\beta$  TCR transgenic mice, endogenous  $\gamma\delta$  TCR is expressed on the same CD4<sup>+</sup>8<sup>+</sup> cells that bear the transgenic  $\alpha\beta$  receptor, indicating that expression of an  $\alpha\beta$  TCR does not necessarily preclude the expression of a  $\gamma\delta$  TCR. Preliminary studies from  $\gamma\delta$ TCR transgenic mice suggest that silencing of TCR  $\gamma$  RNA message may account for  $\gamma\delta$  TCR exclusion in the  $\alpha\beta$  lineage. Experiments to determine the signals inducing the silencing are underway.

To further characterize the extracellular signals that promote  $\gamma\delta$  T cell development, we have used the G8  $\gamma\delta$  TCR transgenic model to analyze the role of MHC in  $\gamma\delta$  development. Although the failure to detect mature transgenic T cells bearing the  $\gamma\delta$  transgene in  $\beta 2m^{-/-}$  mice was previously attributed to a lack of positive selection, a careful analysis of absolute numbers, subsets, and functional competence of thymic and peripheral  $\gamma\delta$  T cells (of further backcrossed mice) revealed that these cells can fully mature in Class I<sup>-/-</sup> mice. Moreover, mixed chimeras



demonstrate that the  $\gamma\delta$  T cells of  $\beta 2m^{-/-}$  origin are deleted in the presence of H-2<sup>d</sup>- bearing cells (presumed to be the positively selecting haplotype). These and other analyses of selection in different MHC haplotypes reveal that there is no requirement for Class I-like molecules for the maturation /development of  $\gamma\delta$  T cells and that the previous results instead may be attributable to a subtle, unsuspected, negatively selecting ligand.

As the controversy on how T cells make the CD4 versus CD8 lineage decision continues, we have developed an *in vivo* model in which we can redirect  $\alpha\beta$  T cells expressing a Class II-specific transgenic TCR from the CD4 into the CD8 lineage by eliminating CD4 expression. The fact that these cells can complete their maturation as CD8 cells in the absence of CD4, indicates that this TCR does not require CD4 for its Class II MHC recognition. Other transgenic  $\alpha\beta$  TCRs also show the same phenomena, but to varying degrees, probably depending on their coreceptor dependence for Class II recognition. These results are important for demonstrating that CD4/CD8 lineage commitment is not strictly dictated by coreceptor usage or by MHC Class specificity. A quantitative signaling model is proposed from these and other results and experimental manipulations to test this hypothesis are in progress.

[E.Schweighoffer, K. Terrence, and B.J. Folkes]

## T Cell Tolerance and Memory

Last year, Dr. Matzinger developed a new model of the immune system based on the idea that its primary function is to discriminate between dangerous and harmless things. This theory made several predictions and this year her laboratory has begun to test some of them. 1) Neonatal tolerance: Female mice can be primed by male dendritic cells or tolerized by male B cells at any time of their life. This result was not predicted by the old 'self-non-self' model which proposed that tolerance to 'self' is set early in life. 2) B cell deficient mice: The theory predicts that B cells are not APCs for naive T cells. To test this, their importance in B cell deficient mice was examined. The T cells in these animals were perfectly able to respond to protein antigens, minor H antigens and parasites. The B cell responses did not differ in any way from those of T cells in B cell sufficient mice. In addition, it has been shown by others that virgin F<sub>1</sub> T cells transferred into a parental mouse cannot use F<sub>1</sub> B cells as APCs. The Matzinger lab is now testing whether antigen presented by B cells is tolerogenic under these circumstances. 3) T cell help for killers. The theory predicts that CD8 killers receive CD4 help via the APC rather than directly from the helper cell. To test this, they have designed an *in vitro* model in which CD4 depleted CD8 T cell populations are unable to generate killers *in vitro* to the male antigen H-Y. They can reconstitute the activity by the addition of small numbers of cloned CD4 helpers, but not with activated dendritic cells. Currently they are treating the dendritic cells in various ways in attempts to overcome the CD4 deficiency.

Another area of progress they have made in tolerance is the completion of a study on maternal tolerance. Using quantitative PCR for the Y chromosome, one occasionally finds male cells in the organs of pregnant mice. This traffic is very sporadic and the cells are rejected by the maternal immune system while the fetus itself is not. Therefore maternal acceptance of the fetus



is most likely produced by the fetal-placental unit, rather than by fetal to maternal traffic. The theory predicts that contact with the healthy fetal decidual cells should generate local tolerance in maternal lymphocytes. This idea is currently being tested.

In the area of T cell memory, the group is studying killer cell memory in mice depleted of T helper cells and B cells. Thus far, killer cell memory lasts 9 months in the absence of T helper cells and 6 months in the absence of B cells. Finally, Lin Yuan has addressed the question of whether the thymic stem cell is precommitted? He has found that the fetal thymus contains uncommitted stem cells at a frequency about ten fold lower than fetal liver. Thus, during fetal development, the commitment to the T cell lineage appears to occur in the thymus, not earlier. [M. Epstein, L. Yuan, E. Bonney, F. DiRosa, and P. Matzinger]

### **Administrative, Organizational, and other Changes**

During the past year, the following people have departed: Dr. Satoru Kitagawa, Dr. David Pham, Kathleen Terrence, Brenda Lawrence, and Randy Faircloth. People that have arrived are Dr. Frank Flomerfelt, Dr. Francesca Macchianini, Dr. Roberta Weiss, Laura Tonnetti, Christine Fleischacker, and Betsy Majane.

### **Honors, Awards, and Scientific Recognition**

Dr. Schwartz is a member of the editorial boards of Science, Immunity, International Immunology, Immunology Today, and Current Opinion in Immunology. During the past year, he was an invited speaker at the European Research Conference on Self and Non-Self Discrimination held in Il Ciocco, Italy, from October 15 to 19, 1994. In January, he was invited to give a seminar on the Molecular Basis of T Cell Costimulation and Anergy at DNAX research laboratories in Stanford, CA. In February, he presented a lecture on Peripheral Tolerance at the American Academy of Allergy and Immunology Annual Meeting in New York City. In March, he was an invited speaker and session chairman at the Keystone Symposium on Dendritic Cells: Antigen Presenting Cells of T and B Lymphocytes held in Taos, New Mexico. In April, he participated in the Human Frontier Science Program Workshop on Molecular and Cellular Mechanisms of Coincidence Detection in the Nervous System: Consequences for learning and memory, held in Strasbourg, France. In June, he gave a lecture in the Department of Immunology at the University of Texas in Houston, Texas. In July, he both gave a lecture on Immunological Tolerance and participated as a guru in the American Association of Immunologists advanced course in Immunology. He also chaired a workshop at the 9th International Congress of Immunology in San Francisco, CA, and gave a lecture in Leiden, The Netherlands, at the Dageraad course on selective modulation of T cell responses in autoimmunity and cancer. Finally, in September, 1995, he presented a Keynote address at the European Conference on Gene Therapy of Cancer in The Strand, London, UK.





Dr. B. J. Fowlkes serves on the editorial boards of *The Journal of Experimental Medicine*, *The Journal of Biomedical Science*, and *The Journal of Immunology*. She also serves on the Committee on the Status of Women for the American Association of Immunologists and this year assumed the position of Chair. Dr. Fowlkes served for the past year on the Search Committee for the Deputy Director for NIH Intramural Research; on the NIAID, DIR Promotion and Tenure Committee; on the NIAID Immunobiology Study Section; and completed a 3-year term as Women Scientists Advisor to the NIAID Scientific Director.

Dr. Fowlkes was invited and is organizing a Keystone Symposium on Lymphocyte Activation to be held in 1996 in Atlanta, GA; and to chair the AAI Special Symposium, *The Changing Face of Science*, and the *Pathways for T Cell Development* workshop, both held at the 9th International Congress of Immunology in San Francisco, CA. Dr. Fowlkes was and invited plenary speaker for the Canadian Society for Immunology Annual Meeting held at Chateau Lake Louise, Alberta, Canada, and for the American Association of Histocompatibility and Immunogenetics Annual Meeting held in Dallas, TX. She was invited to give research seminars to the NIH Immunology Interest Group; the Department of Anatomy/Cell Biology and the Department of Microbiology/Immunology at Wayne State University, Detroit, MI; the Department of Microbiology and Immunology at the University of Texas, Galveston; and the Department of Immunology at the M. D. Anderson Cancer Center in Houston, TX. She was the Graduate Student-Invited Seminar Speaker for the Department of Microbiology at the University of Minnesota, Minneapolis, MI, and lectured in immunology courses at the FAES Graduate School at NIH and in the Department of Microbiology and Immunology at the George Washington University School of Medicine, Washington, DC.

Dr. Matzinger is a member of the editorial boards of Immunobiology, Seminars in Immunology, International Archives of Allergy and Immunology and Current Opinion in Immunology and the scientific advisory boards of the Council for the Advancement of Science Writing and the James A. Baker Center for animal research, Cornell University, and the Life Sciences Fellowships, Princeton, NJ. She is also a regular contributor to "Real Science" a monthly letter for high school students.

During the past year "the Danger model", her new model of the immune system, was featured in Business Week and has been the object of many speaking invitations (see below).

In October, she was invited to teach at the Immunology summer school, Maroochydore, Australia; to speak at The European Research Conference on Self-non-self discrimination in Il Ciocco, Italy; to teach at the European school for Immunology in Naples, Italy; to lecture at the Council for the Advancement of Science Writing's New Horizon's meeting. In November, she gave research seminars at Thomas Jefferson College, PA. Dartmouth College, NH, Stanford University, Palo Alto CA, and the DNAX corporation, Palo Alto, CA. In December she was the keynote speaker at the annual retreat of The Genetics Institute, Boston, MA; gave a research seminar at Case Western University, Cleveland, PA and was an invited discussant at the Memory and MATHematics meeting at Santa Fe, NM. In January, she taught at the Graduate School at NIH and the Office of Education's Immunology elective; she presented her theory at the



Immunex Science Day, New York, NY; gave a research seminar at the University of Virginia, Charlottesville, VA; and was an invited speaker at the Tolerance meeting in Breckenridge, CO and an invited discussant at the Jennifer Jones-Simon Panel On Cancer, Los Angeles, CA. In February, she was an invited speaker at the annual meeting of the American Society of Dermatologists, New Orleans; an invited discussant at the NIH Expert's Panel on Transplantation, she sat on the review board of the Life Sciences Fellowships, Princeton, NJ and gave research seminars at Sloan Kettering Institute, New York, NY, Loyola University, Chicago, IL, and the Mayo Clinic, Rochester, MN. In March, she was an invited symposium speaker at the Dendritic cell meeting, Taos, NM; a workshop chairperson at the meeting on T cell Activation, Taos, NM; and lectured to the Transplantation rounds, NIH. In April, She was the keynote speaker at the annual meeting of the New York Dietetic Association, New York, NY. In May, she taught at the East German International Summer School in Immunology, Friberg, Germany and gave a research seminar at the University of Köln, Köln, Germany. In June, she was an invited symposium speaker at the FASEB meeting on Autoimmunity, Saxton's River, NH. In July, she was an invited symposium speaker at the 9th International Congress of Immunology, San Francisco, CA and taught at the Summer School at Wood's hole, MA. In September, she taught at the 4th International Summer School in Immunology, Prague, Czech Republic; was an invited speaker at the Koning Symposium on Molecular Events in T cell Activation, Leiden, The Netherlands, was an invited symposium speaker at the symposium on the Theory and Science of Immunology, Philadelphia, PA; an invited symposium speaker at the meeting of the Dutch Society for Transplantation, Leiden, the Netherlands; an invited symposium speaker at the meeting of the Austrian Society for Allergology and Immunology 'Immunity versus Tolerance Induction in Peripheral T cells, Vienna, Austria. .

She is a consultant for the Genetics Institute, Boston, MA and Marion Merrel Dow, Kansas City, MO.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00485-09 LCMI

PERIOD COVERED

October 1, 1994 to September 31, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

T Cell Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

Ronald H. Schwartz	Chief, LCMI, NIAID
Satoru Kitagawa	Visiting Fellow, LCMI, NIAID
Jonathan Salo	Commissioned Officer, LCMI, NIAID
Jack Ragheb	Commissioned Officer, LCMI, NIAID
David Pham	IRTA Fellow, LCMI, NIAID
Lynda Chiodetti	Biologist GS-12, LCMI, NIAID

COOPERATING UNITS (if any)

Flow Cytometry and Peptide Synthesis Units, Biological Resources Section,  
 Laboratory of Molecular Structure, NIAID

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Activation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

6.5

PROFESSIONAL:

5.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Over the past several years, we have been studying two phenomena in cloned populations of CD4<sup>+</sup> T lymphocytes referred to as costimulation and anergy. The former entails a 30-100 fold enhancement of interleukin-2 (IL-2) production when signaling through the antigen-specific T cell receptor is supplemented with signaling through the CD28 receptor on the same cell. Anergy is an anti-proliferative state that the T cell enters if it only receives a signal through the antigen-specific receptor. Our recent work has focused on the molecular mechanisms behind these two phenomena. Using IL-2 RNA accumulation and transcription reporter assays, CD28 costimulation was shown to increase the stability of IL-2 messenger(m) RNA and not to enhance the initiation of transcription. An early component of the CD28 effect was nuclear, however, as the enhancement was found in unspliced RNA and for reporter constructs containing the 3' end of the IL-2 gene. The hypothesis we are currently testing is that CD28 costimulation causes the binding or increased stability of RNA shuttle proteins that attach to IL-2 RNAs in the nucleus and accompany them out into the cytoplasm, stabilizing the RNA from degradation in both compartments.

Our previous work on anergy provided evidence that the transactivation of the IL-2 gene by the AP-1 transcription factor was impaired in anergized T cell clones. We have now extended those findings with a variety of new reporter constructs to show that both the distal and proximal AP-1 response elements in the IL-2 enhancer are necessary for anergy induction. Anergy does not affect the induction of new Fos and Jun proteins which make up the AP-1 complex and has only a minor effect on the amount of complex which binds (2-fold less) and its relative affinity for the AP-1 consensus sequence (<2-fold difference). We are currently testing the hypothesis that anergy blocks the induction of AP-1 transactivating activity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI-00613-05 LCMI

PERIOD COVERED

October 1, 1994 to September 31, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

T Cell Subsets

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ronald H. Schwartz	Chief, LCMI, NIAID
Moon Kim	Visiting Associate, LCMI, NIAID
Yongrui Zou	Visiting Fellow, LCMI, NIAID
Sandra Wells	IRTA Fellow, LCMI, NIAID
Frank Flomerfelt	IRTA Fellow, LCMI, NIAID

COOPERATING UNITS (if any)

Lymphocyte Biology Section, Laboratory of Immunology, NIAID, NIH

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Activation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.5

PROFESSIONAL:

4.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to understand the development and functioning of the thymus, both in terms of T cell differentiation and stromal cell environmental support, we have undertaken a molecular approach to identify genes that are uniquely expressed in this organ. Several strategies are currently being explored to accomplish this goal. One is to make cDNA libraries from fetal thymic organ cultures treated with deoxyguanosine and anti-CD45 to remove T cells and other hematopoietic cells. Subtractive hybridization approaches are then undertaken to further enrich for stromal cell cDNAs specific to the organ. The remaining cDNAs are then sequenced to identify new genes. Novel cDNAs are in turn tested by Northern Blot analysis and *in situ* hybridization to check for selective expression in thymic stromal cells. Although this project is still in its early phases, we have already sequenced 288 cDNAs and identified 182 with novel sequences. Two of these appear to be expressed in a thymic cortical epithelial cell line and not in a thymic medullary epithelial cell line. The full sequences of these two cDNAs are now being determined and detection of their expression in thymic sections by *in situ* hybridization is underway.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-00716-02 LCMI

PERIOD COVERED

October 1, 1994 to September 31, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Programmed Cell Death

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Luciano D'Adamino Visiting Scientist and Unit Chief, LCMI, NIAID  
Pasquale Vito Fogarty Visiting Fellow  
Emanuela Lacana Fogarty Visiting Fellow  
Brenda Lawrence GS-7 Technician

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T-cell Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4

PROFESSIONAL:

3

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

T cells recognize peptides bound to MHC molecules using a clonotypic receptor (TCR). Depending on the differentiation state of the T lymphocyte (i.e. responsiveness to a second signal) and/or the context in which the antigenic peptide is presented (i.e. availability of a second signal), this interaction can initiate distinct molecular programs, leading to opposite outcomes (T cell activation, unresponsiveness or death). A precise regulation of these responses results in a system which is both immunocompetent (able to be activated by and eliminate dangerous microorganisms) and self tolerant (able to eliminate or anergize T cell populations bearing TCRs specific for self antigens). The main process through which immunotolerance is achieved involves elimination by programmed cell death (apoptosis) of potentially dangerous T cells (negative selection or clonal deletion). Our main objective is the identification of the genes involved in negative selection and the characterization of the biochemical processes responsible for their functional activation. To this end, we have used a mouse T-cell hybridoma (3D0) as an *in vitro* model. Briefly, we have constructed cDNA libraries into eukaryotic expression vectors using an mRNA source enriched in apoptotic genes. Upon transfection of the libraries, 3D0 has been triggered for apoptosis and recombinant plasmids have been recovered from surviving cells. This sub-library should be enriched in cDNAs able to block death because: 1) expressing in the cells adequate levels of specific antisense RNAs or dominant negative mutants for "apoptotic" genes; and 2) producing "anti-apoptotic" proteins. At the present time we have identified six novel genes whose overexpression in 3D0 inhibits death. Northern blot analysis indicates that they are not ubiquitous and have distinct patterns of tissue distribution. Of interest, four of them are either induced or repressed during T-cells apoptosis both *in vitro* and *in vivo*. For three of them we have cloned the complete coding sequence and expressed the recombinant protein in bacteria to produce specific antisera. Once this reagent will be available, the requirements for the functional activation of these molecules will be studied in more details. In addition, we have isolated genomic clones for two of these genes. They will be used to identify the sequences regulating the transcription of these genes and to generate mice lacking their expression. Once obtained, the mutant mouse will be used as an *in vivo* animal model to study the physiological functions of the genes we have identified.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00486-09 LCMI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT

T Cell Differentiation

PRINCIPAL INVESTIGATOR

B. J. Fowlkes Senior Investigator  
and Section Chief

LCMI, NIAID

Others:

E. Schweighoffer Visiting Fellow

LCMI, NIAID

K. Terrence Howard Hughes Medical Scholar [Oct-Jun]

LCMI, NIAID

F. Macchiarini IRTA Fellow [Jul-Sep]

LCMI, NIAID

E. O'Connell Biologist

LCMI, NIAID

C. Ho Stay-in-school student

LCMI, NIAID

COOPERATING UNITS (if any) Flow Cytometry Section, LMS, NIAID (R. Swofford, C. Eigsti); Animal Care Branch, DIR, NIAID (P. Golway, A. Barnes); Department of Molecular and Cell Biology, University of California, Berkeley (E. Robey); Laboratory of Immunology, NIAID (R. Germain); Laboratory of Developmental and Molecular Immunology, NICHD (K. Ozato, N. Nelson)

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

Thymocyte Differentiation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.25

PROFESSIONAL:

3.0

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) T cell development in the thymus is both genetically programmed and dependent on extracellular signals that serve as checkpoints and/or drive differentiation; such signals appear to promote death, expansion, further maturation, and the divergence of lineages. Our efforts toward defining these signals are concentrated in four areas:

- 1) the mechanism responsible for commitment to  $\alpha\beta$  vs.  $\gamma\delta$  lineage; 2) cellular/molecular interactions required for  $\gamma\delta$  T cell development;
- 3) the nature of interactions inducing positive vs. negative selection; and 4) the mechanisms involved in the CD4/CD8 lineage decision.

During development, productive germline rearrangement must occur in the TCR loci before a TCR heterodimer can be expressed. It has been proposed that commitment to the  $\alpha\beta$  or  $\gamma\delta$  lineage takes place prior to or independent of the rearrangement process. Alternatively, the productive rearrangement and/or expression of a particular TCR heterodimer,  $\alpha\beta$  or  $\gamma\delta$ , could determine lineage choice. In many TCR $\alpha\beta$  transgenic mice, no lymphoid  $\gamma\delta$  T cells develop but, instead, an unusual population of CD4 $^{+}$ 8 $^{-}$  cells appear, bearing the  $\alpha\beta$  transgenic receptor, but with phenotypic and functional properties of  $\gamma\delta$  T cells. The fact that the  $\gamma\delta$  lineage can be "rescued" with an  $\alpha\beta$  TCR indicates that commitment to the  $\alpha\beta$  vs.  $\gamma\delta$  lineage is not dictated by the class of TCR expressed. To further characterize these lineages, we demonstrated that TCR $\gamma$  V to J, as well as TCR  $\delta$  V to J, rearrangements occur in the major  $\alpha\beta$  lineage of both TCR $\alpha\beta$  transgenic and nontransgenic mice; thus, the presence and expression of endogenous or transgenic TCR $\alpha\beta$  does not prevent rearrangement of the  $\gamma$  or  $\delta$  loci. In other  $\alpha\beta$  TCR transgenic mice, endogenous  $\gamma\delta$  TCR is expressed on the same CD4 $^{+}$ 8 $^{-}$  cells that bear the transgenic  $\alpha\beta$  receptor, indicating that expression of an  $\alpha\beta$  TCR does not necessarily preclude expression of a  $\gamma\delta$  TCR. Since inappropriate rearrangements can occur in the wrong lineage, mechanisms must exist to ensure TCR class exclusion. Studies from  $\gamma\delta$  TCR transgenic mice suggest that downregulation of TCR  $\gamma\delta$  RNA message may account for  $\gamma\delta$  TCR exclusion in the  $\alpha\beta$  lineage.

To further characterize the extracellular signals that promote  $\gamma\delta$  T cell development, we have used the G8  $\gamma\delta$  TCR transgenic model. T cells bearing the  $\gamma\delta$  transgene can fully mature in class I $^{+}$  mice, indicating that class I-like molecules are not required for maturation/development of  $\gamma\delta$  T cells. Previous results may be attributable to a subtle, negatively selecting ligand. Whether an MHC-driven positive selection step is necessary for  $\gamma\delta$  development is important to understanding the nature of antigen recognition in mature  $\gamma\delta$  T cells and whether antigen recognition is MHC restricted.

As the controversy on how T cells make the CD4 vs. CD8 lineage decision continues, we have developed an *in vivo* model in which we can redirect  $\alpha\beta$  T cells expressing a class II-specific transgenic TCR from the CD4 into the CD8 lineage by eliminating CD4 expression. The fact that these cells can complete their maturation as CD8 cells indicates that this TCR does not require CD4 for class II MHC recognition. Other transgenic  $\alpha\beta$  TCRs show the same developmental pattern to varying degrees, probably depending on their CD4 coreceptor dependence for class II recognition. These results are important for demonstrating that the CD4/CD8 lineage commitment is not strictly dictated by coreceptor usage or by MHC class specificity; instead, a quantitative signaling model is proposed from these and other results.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-00581-06 LCMI

PERIOD COVERED

October 1, 1994 to September 31, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

T Cell Tolerance and Memory

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Polly Matzinger	Biologist	LCMI, NIAID
Elizabeth Bonney	Medical Staff Fellow	LCMI, NIAID
Francesa Di Rosa	Visiting Fellow	LCMI, NIAID
Michelle Epstein	Visiting Fellow	LCMI, NIAID
Lin Yuan	Visiting Fellow	LCMI, NIAID
John Ridge	Technician	LCMI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Tolerance and Memory Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.2

PROFESSIONAL:

5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The 'DANGER' MODEL: Last year we developed a new model of the immune system based on the idea that its primary function is to discriminate between dangerous and harmless things. We made several predictions and have begun this year to test some of them. 1) Neonatal tolerance: Female mice can be primed by male dendritic cells or tolerized by male B cells at any time of their life. This result was not predicted by the old 'self-non-self' model which proposed that tolerance to 'self' is set early in life. 2) B cell deficient mice: We predicted that B cells are not APCs for naive T cells and tested their importance in B cell deficient mice. The T cells in these animals are perfectly able to respond to protein antigens, minor H antigens and parasites. Their responses do not differ in any way from those of T cells in B cell sufficient mice. 3) B cells in adoptive transfer: Virgin F<sub>1</sub> T cells transferred into parental mouse cannot use F<sub>1</sub> B cells as APCs. We are now testing whether antigen presented by B cells is tolerogenic under these circumstances. 4) T cell help for killer: We predicted that CD8 killers receive CD4 help via the APC rather than directly from the helper cell. To test this, we have designed an in vitro model in which CD4 depleted CD8 T cell populations are unable to generate killers in vitro to the male antigen HY. We can reconstitute the activity by the addition of small numbers of cloned CD4 helpers but not with activated dendritic cells. We are treating the dendritic cells in various ways in attempts to overcome the CD4 deficiency. TOLERANCE 1) Maternal tolerance. Using quantitative PCR for the Y chromosome we occasionally find male cells in the organs of pregnant mice. This traffic is very sporadic and the cells are rejected by the maternal immune system while the fetus itself is not. Therefore, maternal acceptance of the fetus is most likely produced by the fetal-placental unit, rather than by fetal to maternal traffic. We predict, by the 'danger' model, that contact with the healthy fetal decidual cells should generate local tolerance in maternal lymphocytes. 2) Results of tolerance in Tg mice. To test which haplotypes support the development of a Tg TCR derived from an F1 mouse, we created T cell clones from F1 mice, isolated the genes for their antigen specific receptors and generated Tg mice. We now have four transgenics and are breeding them to SCID mice in order to analyse their receptors. T CELL MEMORY: We are studying killer cell memory in mice depleted of T helper cells and B cells. Thus far, killer cell memory lasts 9 months in the absence of T helper cells and 6 months in the absence of B cells. THE THYMIC STEM CELL is it precommitted?. We have found that the fetal thymus contains uncommitted stem cells at a frequency about ten fold fewer than fetal liver. Thus the commitment to T cell development occurs in the thymus, not earlier.



# LABORATORY OF CLINICAL INVESTIGATION

## 1995 Annual Report

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Summary Report  
Laboratory of Clinical Investigation  
October 1, 1994 to September 30, 1995

Stephen E. Straus M.D.  
Chief, Laboratory of Clinical Investigation  
NIAID

## Introduction

The Laboratory of Clinical Investigation (LCI) differs in several regards from all of the other intramural Laboratories within NIAID. Its interests span a wide range of both clinical and basic research problems pertaining to allergic, immunologic, and infectious diseases. In pursuing its mission, the LCI has changed and evolved steadily through the years with the recruitment and departure of staff and in keeping with fundamental research advances and opportunities.

## History

The LCI was formed in 1957 to provide a clinical arm to the NIAID Intramural Program. From that time until 1991, the LCI Chief and the NIAID Intramural Clinical Director were one and the same person. Under the direction of Dr. Vernon Knight, the LCI was comprised largely of virologists who conducted basic and human volunteer studies of respiratory and gastrointestinal viruses and vaccines. In 1968, Dr. Sheldon Wolff assumed the position of LCI Chief and NIAID Clinical Director. Dr. Wolff's philosophy was to establish in the LCI a large number of small units and sections built around promising young allergists, immunologists, and infectious disease physicians who would concentrate on problems relating to inflammatory response and host defenses. Dr. Michael Frank was appointed LCI Chief and Clinical Director in 1978. He expanded his own program of research in complement-mediated disorders and consolidated LCI space into a smaller number of larger research groups.

Dr. Straus was appointed fourth LCI Chief in February, 1991. Numerous changes were made by Dr. Straus in the LCI. Some of the changes were to accommodate the newly formed Laboratory of Host Defenses and the separate Office of the NIAID Clinical Director but, most importantly, to consolidate and redirect the research agenda of the LCI itself. Changes in the LCI were made according to four basic principles. First, the existing LCI sections had grown relatively large and resources needed to be appropriated to accommodate and recruit new young scientists. Second, presaging the newly promulgated NIH-side guidelines for tenure-track scientists, the reality and wisdom of sustaining and supporting all of the, then, "tenure-track" scientists in the LCI was reviewed and firm commitments were made only to those individuals for whom the resources, programmatic needs, and excellence would justify tenure track. Third, the components of the LCI had been relatively isolated one from another because



there was little common ground for collaboration among groups engaged in such widely disparate research areas. If the Laboratory were to sustain efforts in allergy, immunology, and infectious diseases, new recruitment would best be made in disciplines that would bridge the existing LCI interests and draw them closer together. Fourth, there was a major review of all on-going LCI projects and resource commitments to encourage growth toward areas of fundamental cellular and molecular biology and away from some of the more traditional biochemical approaches.

## Organization:

Office of the Chief	In 1995 several changes were made in administrative and secretarial support. Mrs. Carol Penberthy left NIAID to assume a position in NIEHS, Research Triangle Park. Ms. Sara Hursen was recruited from NEI to assume her role as secretary and assistant to the Laboratory Chief. Also, Dr. Adriana Marques was recruited to conduct full-time clinical research studies in collaboration with other LCI Senior Staff. Ms. Janet Dale coordinates the cadre of LCI research nurses.
Allergic Diseases Section	Dr. Dean Metcalfe assumed the position of Head, Allergic Diseases Section. In so doing he extended the reach of his clinical research studies to include asthma by developing a series of new protocols under the direction of Dr. Calman Prussin. Dr. Metcalfe continues to serve as the Director, Allergy and Immunology Program.
Mucosal Immunity Section	Dr. Warren Strober's outstanding work as Deputy Scientific Director, NIAID, ended 1995 with the appointment of Dr. Thomas Kindt as new Director, DIR. For the past two years, a nationwide search has been under way to recruit an additional tenure-track scientist to initiate a new program within Dr. Strober's Section.
Clinical Immunology Section	This Section remains vacant, with the hope of refilling it, as one of LCI's young tenure-track scientists develop sufficiently.
Clinical Mycology Section	Drs. Bennett and Kwon-Chung remained the Senior members of the Section, as they have worked productively side-by-side for about two decades. Dr. Bennett continues not only as Section Head, but also as Head of NIAID's Infectious Disease Training Program Consultation Service. Dr. Kwon-Chung is excellence and growth in studies of molecular aspects of fungal pathogenesis warranted consideration of establishing an independent program under her direction. To this end, documents were submitted before the close of FY 95 to create a new Molecular Immunology Section



with Dr. Kwon-Chung as its Head, and encompassing all the resources and personnel assigned to her while with Dr. Bennett. It is expected that Dr. Bennett and Kwon-Chung will continue their fruitful collaborations.

Medical Virology Section:	Dr. Straus remains Section Head. In FY 1995 Dr. Cohen was awarded Scientific tenure at the NIH. In accord with this and in recognition of his professional growth and achievements, the close of FY 95 brought the establishment under Dr. Cohen's leadership of an independent Molecular Virology Unit. Dr. Cohen was given additional technical support and encouraged to will recruit additional post doctoral fellows. Dr. Robin McKenzie was promoted this year to Medical Officer, GS 13, to conduct clinical studies of hepatitis and herpesvirus drugs and vaccines, and treatment of chronic fatigue syndrome.
Cytokine Biology Unit:	Dr. Joshua Farber was recruited as a tenure-track candidate in 1993 to from this new research unit. He now has four postdoctoral fellows.
Lymphokine Regulation Unit	Also in 1993, Dr. Robert Seder was recruited to the LCI as a tenure-track investigator to head, this new Clinic. He has been allocated a technician and 3 fellows.
Clinical Studies Unit	The complexity of contemporary clinical research demands a level of attention and expertise that no longer permits productive scientists to engage in it casually. The Unit remains vacant, but it is hoped that in the coming year Dr. Marques will assume this position.

## **Current Resources**

The LCI occupies some 35 research modules ranging from 190 to 250 sq. ft. each; six additional office modules ranging from 75 to 250 sq. ft. each are designated for clinical, clerical, and administrative support. There is now a renovated departmental library/conference room. The LCI has one small cold room in the 11C200 corridor; and it shares two other cold rooms on the floor with the Laboratory of Host Defenses, and two large equipment rooms with the Laboratory of Host Defenses and the Laboratory of Immunoregulation. Those large equipment rooms house most of the ultracentrifuges, beta and gamma counters, a phosphorimager and a darkroom. Large common equipment items held within specific LCI Sections include two oligonucleotide synthesizers a 3 channel FACS machine, FPLC machines, and an automated betaplate reader.





## **Operational Overview**

**Administration:** Dr. Straus meets monthly with the Director, DIR and all other Laboratory Chiefs and attends the annual Laboratory Chiefs' retreat. He meets monthly with the Administrative Officer assigned to the Laboratory and, periodically, with the Personnel Officer.

LCI Senior Staff, Administrative Officer, and Head Secretary meet monthly with Dr. Straus to review common issues of space, equipment, resources, recruitment, and budget.

**Budget:** Each Section/Unit develops its own budgetary proposal and reviews it formally with Dr. Straus each spring. The budget is compiled and adjusted by Dr. Straus, and submitted to the Director, DIR. Formal meetings with the Director, and then the Senior Staff occur each fall as the annual budget is allocated by Congress.

**Personnel:** Senior Staff, technicians, and research nurses are relatively fixed resources committed for prolonged time frames to each Section or Unit. Needs for postdoctoral Fellows are reviewed annually with Dr. Straus and committed to the Sections and Units based upon programmatic needs, worthiness of the project, and excellence of the applicant.

**Science:** Each Section or Unit present formal, written reports of its work and plans every spring. Dr. Straus meets formally with each group every fall to review their progress.

LCI Senior Staff and Fellows review their recent work in rotation at research seminars held every Wednesday morning. Each group holds its own weekly research meetings, and most hold weekly journal clubs.

Fellows and staff are encouraged to present their work at national and international meetings, as the restricted travel budget permits. All Fellows are allocated funds for one domestic meeting per year.

Clinical staff round formally three times per week and participate in weekly NIAID Medical Grand Rounds and subspecialty conferences.

## **Clinical Program**

Nearly all of LCI's senior staff are clinically trained and participate in patient care, teaching, and clinical research. Fourteen of LCI's 41 postdoctoral Fellows are in the training programs leading to eligibility for subspecialty boards in Allergy/Immunology or Infectious Diseases.

The LCI staff admit approximately one-fourth of all patients in the 11th floor nursing units and account for 80% of the nearly 2,500 annual visits to the 11th floor clinic.

Clinical Fellows and Physician Assistants carry out much of the primary inpatient care. LCI Fellows and a cadre of four research nurses and one Medical Officer provide primary care, data



collection, and monitoring in the outpatient clinic, all under the direct supervision of LCI Senior Staff.

Major current LCI clinical projects are engaged in studies of the pathogenesis of immunoglobulin deficiency disorders, common variable immunodeficiency, inflammatory bowel disease, mastocytosis, chronic viral and fungal infections, recurrent urinary tract infections, polyclonal lymphoproliferative and autoimmune disorders, and chronic fatigue syndrome. Novel therapies are being explored for mastocytosis, hepatitis, zoster, and candidemia, while vaccines in clinical development in the LCI are directed against genital herpes and cryptococcoses.

### **NIH-Wide and National Commitments**

LCI staff comprise most of the NIH's clinical allergy and much of its clinical infectious diseases expertise. Dr. Metcalfe directs the NIH accredited training program in Allergy and Immunology. Dr. Bennett directs the accredited Infectious Diseases fellowship program. All of the LCI's Senior Staff, excepting Dr. Kwon-Chung, provide clinical service and teaching. LCI staff also serve on many major national and NIH committees and policy boards, including the Recombinant DNA Advisory Committee, the AIDS Data Safety and Monitoring Board, the Infectious Diseases Society of America, the Clinical Immunology Society, the American Academy of Allergy and Immunology, and the American Board of Allergy and Immunology.

### **Honors and Awards**

Dr. Jeffrey Cohen was elected to the American Society for Clinical Investigation this year. Drs. Bennett, Strober, Metcalfe, and Straus were already members of the Society and the Association for American Physicians. Dr. Bennett was elected Vice-President of the Infectious Diseases Society of America. Dr. Kwon-Chung was presented a medal this year by the President of Korea. LCI senior staff undertook several named lectureships and numerous visiting professorships. Dr. Seder was appointed Associate Editor of the *Journal of Experimental Medicine* this past year. LCI staff already serve on editorial boards of *The Journal of Immunology*, *The Journal of Clinical Immunology*, *Clinical and Experimental Allergy*, *Antimicrobial Agents and Chemotherapy*, and *Virology*. Dr. Strober edits *Current Protocols in Immunology*; Dr. Bennett is founding editor of the award-winning textbook *Principles and Practices of Infectious Diseases*. Dr. Straus is an associate editor of *Field's Virology*. With Dr. Bennett, Dr. Kwon-Chung is an editor of two major textbooks of medical mycology.

### **Scientific Accomplishments**

LCI staff were busy and productive this past year with valuable research research contributions emanating from all groups. Several note worthy findings warrant summarization here, as an overview to the ensuing Project reports.



Clinical Mycology Section:	Dr. Geber and Bennett identified <u>Candida glabrata</u> genes (ERG 3, ERG11) resistance to amphotericin B and azoles, commonly used antifungal drugs. Mr. Chang and Dr. Kwon-Chung dissected out yet another key cryptococcal gene that contributes to capsule formation and virulence.
Mucosal Immunity Section:	The Section was particularly productive this year but most noteworthy accomplishments include evidence for the role of OX40 protein in B cell proliferation and immunoglobulin secretion, and the development of a remarkable mouse model of inflammatory bowel disease. Not only do the histologic and clinical features of the process resemble that of Crohn's disease but the immunologic and cytokine profiles of the animals do as well. Drs. Neurath, Fuss and Strober showed that anti-IL-12 can both prevent and treat the bowel disease, suggesting obvious directions for future clinical trials.
Allergic Diseases Section:	Drs. Prussin and Metcalfe initiated a new series of asthma research studies aimed, initially, at least, on characterizing the lymphocyte and lymphokine contributions to reactive airway inflammation. With Drs. Nagataoh, Dr. Metcalfe identified a mutation in c-kit, the mast cell receptor for the SCF growth factor, associated in 4 patients with a particularly aggressive form of mastocytosis.
Lymphokine Regulation Unit:	Dr. Seder developed a murine model of histoplasmosis and showed the ability of IL-12 to initiate an immunologic cascade leading to a speedier resolution of the infection. He also conducted a series of novel studies addressing the role of IL-15 in host responses to HIV infection and tuberculosis.
Cytokine Biology	Dr. Farber showed that the macrophage gamma interferon-responsive chemokine, Mig is synthesized actively in diverse acute infections. He has completed detailed biochemical characterization and purification of Mig protein, and he now turns to creating a Mig knock-out mouse, and identifying the Mig receptor.
Medical Virology Section:	Dr. Cohen, collaborating with Spriggs of Immunex, discovered that the Epstein-Barr virus BZLF2 protein binds to MHC Class II proteins and blocks lymphocyte proliferative responses. With Dr. Heineman, he developed and tested in guinea pigs a new vaccine for genital herpes by inserting the gene for the major HSV2 immunogen, glycoprotein D, into the existing varicella vaccine genome.
	Studies of a new disease, autoimmune lymphoproliferative syndrome culminated this year in the identification in five patients and their family members of heritable mutations in the gene in coding Fas. Autoimmune cytolytic processes, rashes, and lymphoproliferation are consequences of



the failure of Fas-mediated lymphocyte apoptosis. These findings derived from collaborations with Drs. Lenardo and Puck of NIAID and NCHGR, respectively.

## **The Future**

The LCI has been redirected into a group of smaller programs, including three by dynamic young investigators. Recruitment of Dr. Farber to study macrophage gene products brought to the LCI an infectious disease-trained investigator whose bench interests closely relate to those of Drs. Strober and Metcalfe. Dr. Seder, an immunologist, is studying lymphokine responses to three microbes. Dr. Cohen studies the molecular and immunopathogenesis of herpesvirus infections in 1993. These initiatives succeeded in drawing potentially disparate groups of the LCI more closely into a cohesive entity.

In January, 1994, however, the Institute committed to form a new intramural Laboratory of Allergic Diseases and to house it, at least in part, in the remaining space vacated by Dr. Kaliner (1000 sq. ft.) and all of the space currently occupied by Dr. Metcalfe (900 sq. ft.). This will serve to remove all of the allergy research component from the LCI. This demands a further redirection of the LCI program and resources centered solely around fundamental and clinically important aspects of microbial and immunopathogenesis.

Additional staff recruitment, now, can only take place upon the retirement or departure of existing LCI staff. It is expected that the growth and improved productivity of the young, tenure-track scientists in the LCI will justify increasing the resources of one or more of them in the coming three to four years. To serve Dr. Cohen's needs, additional space currently occupied by Dr. Straus' staff will be made available to him. For the others, there remains approximately 600 sq. ft. of "reserve" space in the LCI after creation of the new Laboratory of Allergic Diseases.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI0057-22-LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies on Pathogens Causing Cryptococcosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: K.J. Kwon-Chung, Ph.D. Research Microbiologist, Clinical Mycology  
Section, LCI/DIR/NIAID  
Others: Yun C. Chang, Ph.D. Senior Staff Fellow, Clinical Mycology  
Section, LCI/DIR/NIAID  
Ashok Varma, Ph.D. Microbiologist, Clinical Mycology Section,  
LCI/DIR/NIAID

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID/NIH - Bethesda, Maryland

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Molecular dissection of the genes responsible for the formation of extracellular polysaccharide capsule, the major virulence factor of *Cryptococcus neoformans*, a serious fungal pathogen causing infection primarily in immunocompromised patients: A wild type genomic DNA library of *C. neoformans* B-3501 constructed on a plasmid containing 20 repeats of *C. neoformans* telomeric sequence and *URA5* gene (as a selection marker) was used to complement various *ura5*, *cap* mutants. After phenotypic complementation was observed, the plasmids containing appropriate DNA inserts was rescued by *E. coli*. Subsequent complementation of various *Cap* mutants with the subclones of the insert DNA's indicated that we have cloned two more genes, *CAP64* and *CAP17* in addition to the *CAP59* which was cloned in 1994. When the capsule deficient phenotype was complemented with cloned sequences of *CAP64* or *CAP17*, the originally avirulent mutants regained the same degree of virulence with that found in a highly virulent serotype D strain. DNA sequence analysis revealed that *CAP64* and *CAP17* encode two novel proteins. The *CAP64* deleted strains became acapsular, indicating that *CAP64* is essential for the formation of capsule. Further characterization of the gene *CAP59* indicated that it is clustered with a convergently transcribed ribosomal protein gene, *L27*. By using an inducible promoter (*GAL7*), the missense mutation (glycine to serine) found in the original *CAP59* strain was confirmed as the cause of the acapsular phenotype in the mutant. The two genes were found to be on two separate chromosomes, unlike previous reports that the two loci are closely linked. The *CAP64* gene was found to be joined with a convergently transcribed gene which shares significant similarity to the yeast proteasome subunit, *PRE1*. An *ARS*-like sequence of 1.2kb which allows stable maintenance of an episomal transforming plasmid in *C. neoformans* was isolated. The sequence was obtained from a 6kb *NotI*-*EcoRI* genomic DNA insert cloned from a DNA library of a very stable minichromosomes generated in a *URA5* transformant of *C. neoformans*. The sequence of the 18S rDNA from *F. depauperata*, the only other species described in the genus *Filobasidiella* besides the sexual state of *C. neoformans*, was determined and the sequence was aligned with those of 15 other heterobasidiomycetous fungi in order to place them in a phylogenetic tree. Despite the fundamental differences between *F. depauperata*, which lacks yeast state and animal pathogenicity, and *C. neoformans*, the two fungi were shown to be most closely evolved from the *Tremella* lineage.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 0058-21 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis and Chemotherapy of Herpesvirus Infection in Man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

All the following are members of LCI, NIAID

PI: S.E. Straus, Senior Investigator

Others: J. Cohen, Senior Staff Fellow

R. Kost, Medical Staff Fellow

J. Lekstrom-Himes, Medical Staff Fellow

J. Dale, Research Nurse

M. Nakamura, Medical Staff Fellow

Y. Yi, Visiting Fellow

P. Brunnel, Special Volunteer

R. McKenzie, Medical Staff Fellow

T. Heineman, Medical Staff Fellow

E. Cox, Medical Staff Fellow

P. Hohman, Research Nurse

A. O'Fallon, Research Nurse

K. Wang, Visiting Associate

COOPERATING UNITS (if any)

L. Stanberry, University of Cincinnati; P. Krause, CBER/FDA; R. Hunziker, Transgenic Mouse Facility; D. Burton, Scripps Institute

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

3.8

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to characterize the pathogenesis, natural history and therapy of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. Our clinical emphasis has been on oral and genital herpes and zoster in the normal and the immuno-compromised host. Over the years we established the value, long term efficacy and safety of oral acyclovir for suppression of recurrent genital herpes and more recently oral herpes as well. We are conducting collaborative studies of BVaraU, a new drug for zoster. We continue to seek evidence of persistent acyclovir-resistant HSV infections in immunologically normal individual.

The major basic research thrust of this laboratory has been to define molecular aspects of HSV and VZV latency and pathogenesis. We are examining the role of the HSV 1 and 2 latency-associated transcripts (LAT) in control of virus latency and reactivation. Recombinant viruses deleted for LAT expression and which contain targeted mutations in the LAT promoter are being studied in vitro and in animal models. We have begun to create transgenic mice expressing HSV genes neighboring and/or including the LAT gene. This past year we established mouse ocular models of acute and latent HSV1 and HSV2 infection so that the comparative pathogenesis of these infections can be studied.

Work on VZV latency and gene regulation has concentrated on genes 4, 10, 28, 21, 61, 62, and 63. Because gene 29 is expressed in latency and 28 is not, we are studying the regulation of these two genes. We found that they share a common and overlapping promoter.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00249-14 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Allergic Diseases Section, LCI/NIAID  
Others: Alexandra Worobec, M.D., Medical Staff Fellow, LCI/NIAID  
Hiroshi Nagata, M.D., Ph.D., Fogarty Visiting Fellow, LCI/NIAID  
Arnold Kirshenbaum, M.D., Special Volunteer, LCI/NIAID  
Susetta Finnott, Ph.D., Fogarty Visiting Fellow, LCI/NAID  
Gunnar Nilsson, Ph.D., Special Volunteer, LCI/NIAID

COOPERATING UNITS (if any)

Hematology Section, Clinical Pathology, Clinical Center, Dr. Tannenbaum  
Diabetes Branch, NIDDKD, Dr. Susuki

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseased Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.10

PROFESSIONAL:

2.30

OTHER:

0.80

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mast cell precursor in human peripheral blood is CD34+/Fc $\epsilon$ RI<sup>+</sup>. The number of mast cells arising per CD34+ cell in culture is greater when the CD34+ cells are obtained from patients with mastocytosis with a hematologic disorder compared to normal subjects. This observation led us to search for mutations in the receptor for SCF, c-kit, which would result in enhanced cell proliferation. Using SSCP analysis of PCR products, and genomic DNA analysis, we identified a mutation in c-kit which has been reported in murine and human mast cell lines to result in increased mast cell proliferation. This mutation was associated with mastocytosis in which a hematologic disorder was also present and which exhibited predominantly myelodysplastic features. This mutation was not identified in 67 normal subjects, or in patients with aggressive mastocytosis, and was usually absent in patients with indolent mastocytosis. Interferon alpha-2b was administered to 3 patients with progressive forms of mastocytosis for periods in excess of one year. All patients demonstrated continued progression of disease in spite of interferon alpha-2b administration. Mast cell tryptase in the serum of patients with mastocytosis differs from the tryptase released during anaphylaxis. This difference is recognized by specific monoclonal antibodies and may provide an improved means to follow disease progression and response to therapy. The tryptase found in the sera of mastocytosis is, however, identical to the tryptase found in the sera of patients not experiencing mast cell degranulation, i.e. the "resting tryptase".



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00354-13 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Warren Strober, M.D.	Chief	MIS, LCI, NIAID
Other:	Ivan J. Fuss, M.D.	IRTA Fellow, MIS	MIS, LCI, NIAID
	Markus Neurath, M.D.	Special Volunteer	MIS, LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892-1890

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

2.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of inflammatory bowel disease (IBD) suggest that the basic abnormality consists of an unrestrained immunologic response, not to novel antigen, but rather to common antigens to which the mucosal system is ordinarily unresponsive. To gather data relevant to this hypothesis we have performed extensive studies of control lamina propria T cells. In initial studies we established that as compared to control peripheral blood T cells, lamina propria T cells manifest a greatly reduced response to stimulation via the TCR/CD3 pathway. This was true when cells were studied as a relatively unpurified form or when examined as highly purified CD4+ T cells. Responses via alternative pathways, i.e., via CD2 ± CD28 were also reduced, but not nearly so reduced as via TCR/CD3. In addition, the unresponsive state could be at least partially reversed by cultured cells in IL-2 alone (not IL-2 plus proliferative stimulus) for 24 hours; this suggested that the unresponsiveness was a form of reversible anergy. In further studies, we examined the capacity of peripheral blood and lamina propria T cells to produce various cytokines under the above stimulation conditions. Here we found that both peripheral blood and lamina propria T cells produced relatively low amounts of IL-2, as well as IFN-γ or IL-4, when stimulated via the TCR/CD3 pathway alone, but produced large amounts when stimulated via the CD2/CD28 co-stimulatory pathway. Thus, despite the fact that lamina propria T cells manifest greatly reduced proliferation, they produce as much or more cytokines, as compared to peripheral blood T cells. Finally, we performed studies to elucidate the mechanism of this form of "split" unresponsiveness. In particular, we crosslinked TCR/CD3 T cells under stringent conditions and did indeed induce T cell unresponsiveness (to subsequent stimulation via the TCR/CD3 pathway). However, such cells were also no longer responsive via the CD2 pathway either by proliferation or cytokine production. Thus, the unresponsiveness/responsiveness of lamina propria cells cannot yet be reproduced in culture. Overall, these studies reveal that lamina propria T cells achieve a unique functional state which allows them to act as effector cells in the local mucosal environment.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00356-13 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the regulation of IgA immunoglobulin synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Warren Strober, M.D.,	Chief, Mucosal Immunity Section, LCI, NIAID
Others:	Brian L. Kelsall, M.D.	NRSA Fellow, MIS, LCI, NIAID
	Rolf Ehrhardt, M.D., Ph.D.	Visiting Associate, MIS, LCI, NIAID
	Markus Neurath, M.D., Ph.D.	Visiting Fellow, MIS, LCI, NIAID
	Milan Basta, M.D., Ph.D.	Visiting Associate, MIS, LCI, NIAID
	Eckhard Stuber, M.D., Ph.D.	Special Volunteer, MIS, LCI, NIAID
	Belinda Gray, M.A.	Biologist, MIS, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892-1890

TOTAL STAFF YEARS:

5.5

PROFESSIONAL:

4.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

**Project I:** In ongoing molecular studies of B cell differentiation, we further defined the regulation of the Ig heavy chain 3'α enhancer. Earlier, we showed that the Pax5 transcription factor, BSAP, binds to and represses the activity of this enhancer. We now show that such repression is mediated via regulation of binding of a newly identified Ets family protein, NF-α-P, to a positive regulatory element lying ~50bp downstream of the BSAP binding site. In in vivo footprint analyses, we first showed that the α-P site is occupied only in plasma cells and not in B cells, whereas the reverse is true for the BSAP site. We then showed that when BSAP binding is blocked in vivo by transfection of a triple helix-forming oligonucleotide that binds to the BSAP site, an α-P footprint appears and Ig heavy chain transcription is increased. Finally, we showed that the triple helix-forming oligonucleotide also increases enhancer activity of a transfected 3'α enhancer construct in B cells, but only when the α-P site is intact. Thus, BSAP negatively regulates the 3'α enhancer by blocking the binding of NF-α-P to a positive regulatory element, αP. **Project II:** In these studies we defined the role of the T cell/B cell interaction mediated by OX40L-OX40 on B cell differentiation. In initial studies we showed that crosslinking of OX40L on CD40L (or anti-ID-dextran) stimulated B cells, or both, results in enhanced B cell proliferation and Ig secretion, independent of added cytokines. We then showed that OX40L crossbinding results in the downregulation of the above-mentioned BSAP which, in turn, leads to occupation of the α-P binding site in the 3'α enhancer. Thus, in this novel pathway of T cell dependent B cell differentiation, the cell surface interaction acts through 3'α enhancer activation. **Project III:** In a broad study of T cell differentiation and regulation in Peyer's patches (PP) we have continued our examination of PP dendritic cells (DC). Using immunoperoxidase staining of frozen sections of murine PP, we demonstrated the presence of a dense layer of cells with DC morphology, just beneath the PP dome epithelium, that stained with antimurine CD11c (mAb IV418) an antibody associated with DCs. Such cells were distinct from a second population of DCs that were also stained with the NLDC-145 and M342 mAb, intracellular DC markers associated with more differentiated DCs. In functional studies we showed that PP dendritic cells can be loaded with oral administered antigen to present to T cells bearing receptors for the fed antigen. Finally, we showed that PP DCs, when stimulated with activated T cells, produce significantly more IL-12 than spleen DCs, thus accounting for a previous finding that PP DCs induce T cells producing 5-10 fold more IFN-γ than spleen DCs. Overall, these studies show that PP DCs take up antigen that enters the PP (probably via M cells) and then present the antigen to T cells to induce Th1-type T cell responses.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

ZOI AI 00430-11 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Varicella-Zoster Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J. I. Cohen	Senior Investigator, LCI, NIAID
S. Straus	Chief, LCI, NIAID
R. Kost	Clinical Associate, LCI, NIAID
M. Moriuchi	Bio. Lab. Tech., LCI, NIAID

COOPERATING UNITS (if any)

S. Triezenberg, Michigan State University

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.9

PROFESSIONAL:

1.4

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1) Primary infection with varicella-zoster virus (VZV) causes chickenpox, and reactivation of the virus from latency results in zoster. The goals of this project are to identify and determine the function of VZV genes that are expressed during active infection and during latency in the body.

2) VZV genes that are expressed during active infection and regulate viral gene expression in vitro are being studied. Analysis of VZV gene products during acute infection indicate that five VZV genes, ORF4, ORF10, ORF61, ORF62, and ORF63, regulate the expression of other VZV genes in vitro. Two cellular proteins (Oct1, HCF) have been shown to interact with a VZV protein (ORF10) to activate the ORF62 gene. These cellular proteins have been shown to bind to a specific sequence on the VZV ORF62 promoter. A different cellular protein (USF) has been found to interact with another VZV protein (ORF62) to activate expression of two VZV genes (ORF28, ORF29). The USF protein binds to a specific sequence located between the ORF 28 and 29 genes.

3) Critical domains required for the activity of two VZV genes (VZV ORF4 and ORF61) have been identified. A domain near the amino terminus of ORF4 is required to activate expression of other VZV genes. This domain can functionally substitute for its corresponding homolog in a herpes simplex virus protein. A domain in ORF61 is found in the RING finger family of proteins and is required for functional activity of ORF61 protein. The transcription activation domain of ORF10 has been identified at the amino terminus of the protein. This activation domain has been found to share structural features with its herpes simplex virus VP16 homolog.

4) Central nervous system tissues obtained from individuals who have recovered from chicken pox infection in the distant past are being studied to determine which VZV genes are expressed while the virus is dormant in the human body. Analysis of trigeminal ganglia from human cadavers without active evidence of VZV infection indicates that two VZV genes, ORF29 and ORF62, are expressed during latency in non-neuronal (satellite) cells, while other viral genes (ORF10, ORF61) are not expressed.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00432-11 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of immune responses in humans and non-human primates.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI, NIAID

Others: Monica Boirivant Visiting Fellow, MIS, LCI, NIAID  
 Yohko Murakawa Visiting Associate, MIS, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH Clinical Center, Bethesda, Maryland 20892-1890

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

2.25

OTHER:

0.

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

L-selectin (LAM-1/LECAM-1) the selectin expressed on leukocytes mediates a number of leukocyte-endothelial interactions, including the binding of lymphocytes to high endothelial venules (HEV) of peripheral lymph nodes and Peyer's patches, the binding of neutrophil to endothelial cells and the binding of all leukocytes to inflamed venules. Recently we showed that L-selectin ligation influences the T cell activation process: immobilized Leu-8 mAb influences the anti-CD3-mediated proliferation of T cells and co-immunoprecipitates molecules in the TCR/CD3 complex. In the present study, we provide further evidence of the involvement of L-selectin in signal transduction by showing that ligation of L-selectin augments phosphoinositol (PI) hydrolysis induced by anti-CD-3 and accelerates and enhances tyrosine phosphorylation induced by anti-CD3. In further studies, we examine the signaling function using immunoprecipitation techniques aimed at identifying molecules associated with L-selectin under various conditions. First, we showed that in resting cells--metabolically labeled with 35-S methionine and 35-S cysteine and then exposed to non-dissociating detergent lysing agents, anti-L-selectin coimmunoprecipitates both a 94 kD and a 210 kD molecule identified on SDS-PAGE. These molecules are likely to be intracytoplasmic (non-surface) molecules, since they are not detected in lysates derived from surface-labeled cells. Second, we showed that in metabolically labeled and anti-L-selectin clustered cells, anti-L-selectin coimmunoprecipitates a 210 kD molecule which, in an in vitro kinase assay, takes up 32-P. Furthermore, in the same cells activated with Con A, anti-L-selectin coimmunoprecipitates a 150 kD molecule as well as the 210 kD molecule that take up 32-P. These data establish that L-selectin is a signal transduction molecule that associates with several other molecules, at least some of which have tyrosine kinase activity. We hypothesize that the latter are critical for the ability of L-selectin to signal cells directly or via TCR/CD3. In additional studies, we sought to determine if L-selectin-mediated cell signaling has functional consequences other than an effect on proliferation. In particular, we investigated the role of L-selectin in T cell cytokine production. We demonstrated utilizing quantitative RT-PCR, that L-selectin crosslinking of anti-CD3 activated CD4+ T cells leads to an approximately 10-fold higher steady state IFN-gamma mRNA levels at an early time point (6 hours), but not at later time points (12 and 24 hours) and this effect was seen when L-selectin and TCR/CD3 ligations were applied simultaneously or sequentially. L-selectin ligation alone, in the absence of anti-CD3 signalling did not induce an INF-gamma mRNA increase. The above results are relevant to T cell-endothelial interaction occurring in the vasculature at sites of inflammation, as well as to immune response at particular sites.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00470-10 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chronic Epstein Barr Virus Infection and Chronic Fatigue Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

All the members below belong to LCI, NIAID

PI: S.E. Straus - Senior Investigator J. Cohen - Senior Staff Fellow  
J. Dale - Clinical Research Nurse J. Lekstrom-Himes - Clinical Assoc.  
R. McKenzie - Medical Officer A. O'Fallon - Clinical Res. Nurse  
W. Strober - Senior Investigator

COOPERATING UNITS (if any)

J. Grafman, M. Hallett, A. Samii, E. Wasserman (LNM, NINCDS),  
H. Rotbart (Univ. of Colorado), F. Hayden (Univ. of Virginia),  
M. Demitrack (Univ. of Michigan), D. Garcia-Borregeros, M. Altemus, P. Gold, N. Rosenthal (NIMH), D.  
Garcia, G. Chrousos (NICHD), Scott Fritz (PRI, FCRF), T. Fleischer (CC, NIH)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.25

PROFESSIONAL:

3.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to characterize severe chronic infections with Epstein Barr Virus (EBV) and other lymphoproliferative disorders, and to elucidate multiple aspects of the chronic fatigue syndrome which was, earlier, considered to be related to EBV infection. To date this research project has involved over 300 patients. Included are 9 patients who were diagnosed with severe chronic EBV infections on the basis of clinical, histological, molecular and serologic features. We continue to examine immunologic features of patients with severe chronic EBV-associated lymphoproliferation and explore treatments. Acyclovir, alpha and gamma interferons proved of little value, but immunosuppressive therapies are being used with good long-term results.

Detailed immunologic, neurologic, endocrinologic and psychologic studies are being conducted on selected patients with chronic fatigue. To date, we still have no consistent laboratory abnormality that permits a clear diagnosis of the chronic fatigue syndrome. A series of earlier studies of the pituitary-adrenal suggested deficient central CRH release. Since CRH induces CNS arousal, these neuroendocrine findings suggest a new mechanism whereby the lethargy of Chronic Fatigue Syndrome patients may be explained. We are pursuing these observations in a new series of studies and a fresh patient cohort. Arginine-Vasopression infusions were given to CFS patients and controls to stimulate and test the HPA axis and the data are under analysis, 62 of 70 desired were enrolled in a placebo-controlled trial of hydrocortisone treatment. It should permit us to test the hypothesis that corticosteroid deficit leads to symptoms. We completed a study showing the absence of seasonality in CFS symptoms, further distinguishing the it from Seasonal Affective Disorder. We recognized discrete abnormalities in CFS patient lymphocyte phenotype and in vitro responsiveness to mitogens in patterns suggesting mild immune activation. We noted a reduction in naive T Cells and an increase in memory T cell bearing adhesion markers. We have begun to pursue these findings and related immune studies in populations a new CFS patint cohort and in patients with acute influenza. A vigorous, multidisciplinary approach to the syndrome continues.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00513-08 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Mast Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Allergic Diseases Section, LCI/NIAID  
Others: Peter Bianchine, M.D., Medical Staff Fellow, LCI/NIAID  
Jaroslaw Dastyh, Ph.D., Fogarty Visiting Fellow, LCI/NIAID  
Susetta Finotto, Ph.D., Fogarty Visiting Fellow, LCI/NIAID  
Arnold Kirshenbaum, M.D., Special Volunteer, LCI/NIAID  
Karin Hartmann, M.D., Special Volunteer, LCI/NIAID  
Harissios Vliagoftis, M.D., Visiting Associate, LCI/NIAID

COOPERATING UNITS (if any)

Others: (cont.)

Gunnar Nilsson, Ph.D., Special Volunteer, LCI/NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.50

PROFESSIONAL:

4.30

OTHER:

0.20

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mast cells attach to connective tissue components following aggregation of high affinity receptors for IgE (Fc<sub>ε</sub>RI) or low-affinity receptors for IgG (FcγRII/III) on the membrane surface. This process is mediated by integrins. FcR induced adhesion is transient and occurs through "inside-out signaling". Signaling through FcR that leads to adhesion requires participation of the gamma chain.

Mast cells spontaneously adhere to vitronectin. This event is followed by the phosphorylation of intracellular proteins including focal adhesion kinase (FAK). FAK is also phosphorylated after aggregation of Fc<sub>ε</sub>RI, or after the addition of c-kit ligand, also termed stem cell factor (SCF). The autophosphorylation activity of FAK is also increased when mast cells are activated by SCF, by adhesion to vitronectin, or following Fc<sub>ε</sub>RI aggregation.

IL-3 dependent mast cells undergo apoptosis following removal of IL-3. This is prevented by the addition of SCF. Addition of TGF-beta prevents this SCF-mediated rescue, but does not effect IL-3-dependent proliferation. Mast cell apoptosis is accompanied by down-regulation of Bcl-2.

Mouse bone marrow-derived mast cells respond to both IL-3 and c-kit ligand and are inhibited by m-CSF, GM-CSF, and γ-IFN. Mouse peripheral blood mononuclear cell-derived mast cells respond only to c-kit ligand.

Human bone marrow-derived or peripheral blood-derived human mast cells may be cultured from a CD34+ Fc<sub>ε</sub>RI cell population in the presence of SCF. CD34 positivity is rapidly lost in culture, while the cell population becomes Fc<sub>ε</sub>RI+. Mast cell outgrowth is inhibited by IFN-γ, but not IFN-α2b, and this inhibitory effect is not abrogated by IL-4.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 AI 00548-07 LCI
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Prevention of Genital Herpes Simplex Infection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  PI: S.E. Straus - Senior Investigator, LCI, NIAID R. McKenzie - Medical Officer, LCI, NIAID A. O'Fallon - Research Nurse, LCI, NIAID R. Kost - Medical Staff Fellow, LCI, NIAID J. Lekstrom-Himes - Medical Staff Fellow, LCI, NIAID M. Nakamura - Medical Staff Fellow, LCI, NIAID E. Cox - Medical Staff Fellow, LCI, NIAID P. Hohman - Research Nurse, LCI, NIAID		
COOPERATING UNITS (if any)  R.L. Burke, M. Tigges, A. Langenberg, C. Dekker, (Biocine, Inc., Emeryville, CA) L. Corey, (University of Washington, Seattle)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Medical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.5	PROFESSIONAL: 2.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Having completed over a decade of work on antiviral treatment of genital herpes, we turned to studies of disease prevention and immunotherapy. We completed an open 2 year phase 1 assessment of a new recombinant HSV-2 glycoprotein D vaccine in alum in 24 adults, some with and without prior HSV-1 and/or 2 infection. Vaccinations of 30 ug or 100 ug were given at 0, 1, 2, and 12 months and were associated with minimal local or systemic reactions. The vaccine induced excellent primary antibody in cellular immune responses and augmented preexisting humoral and cellular responses significantly. We studied antibody and T cells from previously uninfected subjects and showed that the gD2 epitopes that the antibody and cell recognize correspond to those in genital herpes patients. On the basis of these excellent responses we enrolled patients with recurrent genital herpes into a placebo-controlled vaccine trial in collaboration with the University of Washington. The goal was to determine whether boosted immunity leads to less frequent recurrences. Upon completing the study we documented that of 98 subjects, those who received the gD2 vaccine in alum experienced 1/4 - 1/3 fewer recurrences than those receiving alum alone. This is the first trial to demonstrate efficacy in the immunotherapy of a chronic viral infection. Rates of disease improvement, though, were lower than would be expected with acyclovir suppressive therapy so we began a dose seeking study and an efficacy study using gD2 combined with gB2 in a lipid adjuvant MF59. This was completed in FY1993. Based on the data we initiated 2 further controlled trials, one for immunotherapy, the other for prophylaxis. We completed enrollment of the immunotherapy trial in which patients are vaccinated with gB2/gD2/MF59 at 0, 2, 12, and 14 months and followed for recurrence of rats and severity for 18 months. The study will close in October 1995. We also completed enrollment of a multicenter study of gB2/gD2/MF59 for prevention of genital herpes. The study will end in 1996.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00590-06 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Primary Immunodeficiency Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI, NIAID

Others: Thomas A. Selvaggi, M.D. Clinical Associate, MIS, LCI, NIAID

Kevin Chua, B.S. Biologist, MIS, LCI, NIAID

COOPERATING UNITS (if any)

Laboratory of Host Defenses, NIAID (Harry L. Malech, M.D.)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892-1890

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Studies in our laboratory have focused on defining the nature of the B cell and T cell defects in Common Variable Immunodeficiency (CVI), a primary acquired human immunodeficiency state characterized by hypogammaglobulinemia and impaired functional antibody responses. Previous studies of purified B cells of patients with CVI show that although the cells have a normal capacity to proliferate, they manifest differentiation defects at multiple levels. Thus, as compared with normal B cells, circulating CVI B cells contain reduced numbers of sIgG+ and sIgA+ cells with a commensurate increase in sIgM+ B cells, suggesting an in vivo defect in isotype switch. In addition, they fail to undergo differentiation into immunoglobulin-producing cells. We now have shown that these defects are associated with the ability of CVI B cells to upregulate and sustain high level surface expression of a critical T cell ligand, B7-2. We have found that CVI B cells, under a variety of stimulatory conditions manifest premature upregulation of B7-1, a surface molecule now thought to inhibit B cell-T cell interaction. These abnormalities of cell surface B7 expression are a likely explanation of the fact that CVI B cells are relatively poor antigen presenting cells (APC). Thus, purified CVI B cells are less efficient APC when compared to normal B cell of PHA-stimulated T cells using IL-2 as a read-out of T cell stimulation. Moreover, this deficiency is corrected by the addition of anti-CD28 antibody, i.e., an antibody that replicates the function of B7. Overall, these data provide evidence that B cell function in CVI is abnormal in a way that is quite separate from the known immunoglobulin production deficit. A second area of focus in immunodeficiency concerns X-linked agammaglobulinemia (XLA), another major humoral immunodeficiency syndrome. In this disease, B cell development is arrested at the pre-B cell stage and there is an absence of mature B cells. This abnormality results from mutation in a gene, the btk gene whose product is a key tyrosine kinase in B cell activation. In the present study we have prepared a retroviral vector containing a btk cassette and have used this vector to transfer btk into NIH 3T3 fibroblasts. If this vector is producing btk protein, as determined by tyrosine kinase activity, we will be in a position to transfect patient CD34+ cells with the retroviral vector.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

01 AI 00607-05 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Cytokine Gene Expression in Mast Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Allergic Diseases Section, LCI/NIAID

Other: Chad Oh, M.D., Medical Staff Fellow, NCI/NIAID

Jaroslav Dastych, Ph.D., Fogarty Visiting Fellow, LCI/NIAID

Vanitcha Rumsaeng, M.D., Visiting Associate, LCI/NIAID

Harissios Vliagoftis, M.D., Visiting Associate, LCI/NIAID

Gunnar Nilsson, Ph.D., Special Volunteer, LCI/NIAID

COOPERATING UNITS (if any)

Mucosal Immunity Section, LCI, NIAID (Markus Neurath)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.90

PROFESSIONAL:

2.50

OTHER:

0.40

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Promoter analysis of the TCA3 chemokine gene identified minimal promoter sequences contained within the 0.082 kb upstream region of the TCA3 gene with a putative enhancer NF-kB element between -0.324 kb and -0.136 kb and a putative inhibitory element between -1.324 kb and -2.0 kb upstream from the transcription start site. Functional assessment of the region between -1.324kb and -2kb demonstrated that there are at least two negative regulatory elements (NREs) that control the transcription of the TCA 3 gene in activated mast cells. Both NREs inhibited the activity of a CD20-CAT construct in mast cells, independent of cell activation. One NRE was similar to a putative silencer motif in the  $\alpha 2b$  integrin gene. The second NRE was novel and was characterized by a CT-rich sequence. Mast cells transfected with a TCA3-CAT construct and co-cultured with activated lymphocytes, exhibited an increase in CAT expression. Supernatants from activated lymphocytes had no effect. This data demonstrates that activated lymphocytes have the ability to induce the promoter of the TCA3 gene in mast cells through cell-to-cell contact. The trkA tyrosine kinase receptor functions as a signal transducing receptor for nerve growth factor (NGF). By Northern blot analysis we have found that HMC-1 cells, (a human mast cell line) express mRNA for trkA, but not for other high affinity neurotrophin receptors, nor the low affinity neurotrophin receptor p75. Expression of trkA protein was demonstrated by Western blot and immunohistochemistry. The trkA receptors expressed on HMC-1 are functional in that NGF significantly increased the mRNA levels for the early response gene *c-fos*.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00650-04 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Treatment of Chronic Hepatitis B Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.E Straus - Senior Investigator, LCI, NIAID  
R. Mc Kenzie - Medical Officer, LCI, NIAID

COOPERATING UNITS (if any)

J. Hoofnagle (NIDDK)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.25

PROFESSIONAL:

0.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been exploring the potential antiviral efficacy of nucleoside analogues for treatment of chronic hepatitis B virus infection. Initially we explored the drug FIAC for inhibition of CMV infection in AIDS patients. Antiviral activity was not seen at tolerated doses and studies turned to the drug's metabolic derivative, FIAU. Studies showed intolerance at high doses but excellent tolerance and profound inhibitory activity at lower doses against hepatitis B virus (HBV). The initial studies involved HIV positive patients with co-existing chronic HBV infection. Profound and even permanent reductions in circulating HBV DNA and antigens were achieved with oral FIAU at daily doses of 0.1, -.5, or 1 mg/kg for 14 days. We then turned to a broader dose modification study in normal patients with chronic HBV infection. Studies compared antiviral efficacy at .05, 0.1, 0.25 and 0.5 mg/kg per day for 28 days. We noted profound inhibition of HBV infection even at the lowest doses and in the absence of any substantial side effects. By 9 months after treatment 9 of 24 patients had lost HBV DNA; 2 of which lost HBeAg. Based on these findings additional HIV-negative chronic HBV patients were enrolled in a randomized study comparing 0.1 and 0.25 mg/kg per day for 6 months to determine long term safety and efficacy. The trial was terminated 6/26/93 because of the emergence of life-threatening toxicity in the study. The past year of work entailed long term follow up of the study patients and a multi-faceted exploration of the cellular and biochemical bases for the toxicity. We also began studies of additional, newer nucleoside analogs that already have considerable evidence of safety in other disease settings. We obtained IND to study famciclovir in combination with recombinant interferon. Open studies of 3TC are also being considered.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 A1 00654-04-LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular genetics, biochemistry and therapy of *Candida albicans*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Bennett, M.D. Head, Clinical Mycology Section, LCI/DIR/NIAID

Others: Antonia Geber, M.D. Guest Researcher

COOPERATING UNITS (if any)

Christopher A. Hitchcock, Ph.D. - Pfizer Central Research - Sandwich, England

Douglas J. Ward, M.D. - Washington, DC

Virginia Kan, M.D. - Assistant Professor of Medicine - VAMC - Washington, DC

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID/NIH - Bethesda, Maryland

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The *C. glabrata* *ERG11* gene, encoding the cytochrome P-450-dependent  $\alpha$ -14 demethylase, was deleted in order to assess the effect on sterol synthesis, aerobic viability and azole susceptibility. A mutant in which both *ERG3* and *ERG11* was deleted was aerobically viable and accumulate 14methylfecosterol. A mutant in which only *ERG11* was deleted was not aerobically viable but gave rise to an aerobically viable revertant which accumulated both lanosterol and obtusifoliol. This *ERG11* mutant had an intact *ERG3* gene and increased *ERG3* message on Northern analysis. This contrasts with *S. cerevisiae*, in which aerobically viable *ERG11* revertants have no functional *ERG3* gene. The *ERG11* revertant and the *ERG3*, *ERG11* mutant were resistant to azoles, slightly more resistant to amphotericin B and more readily killed by human neutrophils or hydrogen peroxide.

We have enrolled four patients in our study of oropharyngeal candidiasis in AIDS patients. One of the four had increased azole resistance in both his *C. albicans* and *C. glabrata* isolates at time of relapse. The mechanism by which resistance occurred is under study.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00655-04-LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Pathogenic Fungi

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Bennett, M.D. Head, Clinical Mycology Section, LCI/DIR/NIAID  
 Others: Peter R. Williamson, M.D., Ph.D.  
 Medical Staff Fellow, Clinical Mycology Section, LCI/DIR/NIAID  
 Steven D. Salas, M.D.  
 Medical Staff Fellow, Clinical Mycology Section, LCI/DIR/NIAID  
 David Kruse, Ph.D.  
 Postdoctoral Fellow, Clinical Mycology Section, LCI/DIR/NIAID

COOPERATING UNITS (if any)

J. C. Edman, Ph.D. - University of California at San Francisco

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID/NIH - Bethesda, Maryland

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An isolate of *Cryptococcus neoformans* in which the structural gene for diphenol oxidase (CNLAC1) has been disrupted was crossed with a wild type and the F1 progeny studied for virulence. A F1 strain which produced diphenol oxidase (DPO) killed mice whereas a strain which did not produce DPO did not cause lethal infection. However, complementation of the CNLAC1 deletant with CNLAC1 produced a strain which was not lethal for mice, even though the isolate produced DPO. All these strains were the same mating type (a) and had the uracil auxotrophy of the CNLAC1 deletant restored by either backcrossing or transformation. The deletant was found to be missing not only the 5' end of the gene and the URA5 gene used for positive selection, but also approximately 4kb of upstream sequences. Although no transcript has been detected from the area of the 5' deleted sequences as yet, it is possible that sequences coding for regulatory or structural genes are present in this area. The upstream deletion might have decreased virulence apart from disrupted CNLAC1 gene. Another experiemnt which supports the nonessentiality of the CNLAC1 gene for virulence is that a mutant (mel2) obtained from J.C. Edman did not produce DPO but was able to kill mice. When transformed with CNLAC1 the isolate made DPO but did not have a substantial increase in virulence.

CNLAC1 was used to integratively transform *Pichia pastoris*. The expressed and secreted protein was partially purified on phenylsepharose and DEAE chromatography. The expressed protein was contained in a fraction containing at least two proteins, based on agar gel precipitin bands. The mixture was heavily glycosylated, containing 41% mannose. Deglycosylated enzyme was inactive. On TSK 4000 HPLC chromatography, DPO enzymatic activity was approximately 100-130 kd and had a pI of less than 4.5 on isoelectric focusing. When compared with a DPO transcript size of 1.3-1.8 kb, it seems likely that DPO is being expressed as a heavily mannosylated protein with a heterodisperse molecular weight.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00657-04 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies on *Aspergillus* Species

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K.J.Kwon-Chung, Ph.D. Research Microbiologist, Clinical Mycology  
Section, LCI/DIR/NIAID  
Others: Mark J. Parta, M.D. Medical Staff Fellow, Clinical Mycology Section,  
LCI/DIR/NIAID  
Yun C. Chang, Ph.D. Senior Staff Fellow, Clinical Mycology Section,  
LCI/DIR/NIAID

COOPERATING UNITS (if any)

Ronald G. Washburn, M.D. - Bowman Gray School of Medicine

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID/NIH - Bethesda, Maryland

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Identification of the genes associated with virulence in *Aspergillus* species: *Aspergillus* species cause substantial morbidity and mortality in humans principally among those compromised by chronic granulomatous disease (CGD) or prolonged neutropenia. Although it is known that aspergillosis is caused by inhaled conidia, little is known about the precise events and interactions with the host that lead to successful survival and propagation of the fungus. *Aspergillus* conidia (spores) are known to be covered by a layer of hydrophobic proteinaceous fascicles called rodlets. The rodlet layer is believed to be responsible for the hydrophobic nature and the dispersibility of the spores. It has been shown that resting conidia of *A. fumigatus* with intact rodlet layers are killed much less effectively by human neutrophils than the swollen, rodletless hydrophobic conidia. It has also been shown that hydrophilic conidia are more susceptible to neutrophil oxidative products and rabbit neutrophil cationic peptides. In addition, macrophages are also less able to kill hydrophobic spores than hydrophilic spores. In the previous year we have cloned the *HYP1* gene responsible for the formation of rodlet layer in *A. fumigatus* and showed that it is expressed in *A. nidulans* another *Aspergillus* pathogen primarily affecting CGD patients. The second gene responsible for the hydrophobicity of *A. nidulans* conidia was cloned by Stringer et. al., recently. This year we studied the effect of hydrophobins on fibrinogen binding and alternative complement pathway-mediated opsonization using hydrophobin gene disrupted mutants of *A. nidulans*. The results suggested optimal fibrinogen binding requires an intact hydrophobin layer of conidia and that competition takes place between fibrinogen and complement-uptake on conidial surfaces. These results indicate that hydrophobins on the conidial surface play an important role in the opsonophagocytosis of *Aspergillus* conidia.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

ZOI AI 00674-03 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Varicella-Zoster Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J. I. Cohen	Senior Investigator, LCI, NIAID
T. Heineman	Medical Staff Fellow, LCI, NIAID
E. Cox	Medical Staff Fellow, LCI, NIAID
J. Ross	Medical Staff Fellow, LCI, NIAID

COOPERATING UNITS (if any)

J. Hooks, National Eye Institute  
L. Stanberry, Children's Hospital, Cincinnati, OH

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20982

TOTAL STAFF YEARS:

3.3

PROFESSIONAL:

3.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

1) Varicella-zoster virus (VZV) is the etiologic agent of chickenpox and herpes zoster. We have developed a system to mutate individual genes or to insert foreign DNAs in the VZV genome. These mutant viruses are being assayed for their ability to grow in cell culture or in VZV animals models.

2) Mutations have been engineered into the VZV genome that result in virus that is unable to express the viral glycoprotein V, ORF1 protein (expressed on the surface of infected cells), ORF47 protein kinase, or ORF10 protein (a transactivator). Viruses that are unable to express each of these genes grow at similar rates as the parental virus in cell culture. VZV ORF10 is the functional homolog of herpes simplex virus VP16. While VP16 is essential for replication of herpes simplex in vitro, deletion of ORF10 did not alter the growth of VZV in vitro. Comparison of phosphorylation products in cells infected with parental or ORF47 mutant VZV indicates that the ORF47 protein kinase is responsible for phosphorylation of several proteins in infected cells. Guinea pigs will be inoculated with selected VZV mutants to determine if the viruses have lost the ability to establish latency or reactivate from the central nervous system. Such viruses might be useful and candidate vaccines.

3) The *E. coli* beta-galactosidase gene, has been inserted into the VZV genome, and the resultant virus produces plaques that stain blue with X gal. Intraocular inoculation of guinea pigs with VZV expressing beta-galactosidase results in a chronic uveitis with mononuclear inflammatory cells in the vitreous and VZV present in the ciliary body and retina. Virus was detected in the eye at least two months after inoculation. This model should be useful for studying the role of individual viral genes during infection in vivo.

4) The herpes simplex virus (HSV) glycoprotein D (gD) gene, encoding a major neutralizing antigen, has been inserted into VZV. The resulting virus expresses HSV gD on its surface, and cells infected with the virus express gD on their cytoplasmic membrane. Inoculation of guinea pigs with VZV expressing HSV gD, followed by challenge with intravaginal HSV, resulted in production of neutralizing antibodies to HSV and in reduced severity of genital herpes. A second generation vaccine has been constructed that expresses HSV gD and glycoprotein B and will be tested in animals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 AI 00680-03
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Novel Cytokines From Activated Macrophages		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Joshua M. Farber	Investigator LCI/NIAID
Others:	Fang Liao	Visiting Fellow LCI/NIAID
	Hwang-Ho Lee	Visiting Fellow LCI/NIAID
	Ronald Rabin	Clinical Associate LCI/NIAID
COOPERATING UNITS (if any) Philip Murphy, LHD, NIAID; John Yannelli, SB, NCI		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Cytokine Biology Unit		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The overall goal of the project is to identify and characterize macrophage products of potential importance in immune and inflammatory responses in order to manipulate these responses for clinical benefit.</p> <p>Differential screening of a cDNA library prepared from a mouse macrophage-like cell line led to the identification of two mRNA species, designated Mig and Crg-2 that encode previously undescribed members of a newly-defined family of small secreted proteins, termed chemokines. Crg-2 is likely the murine homologue of the human chemokine IP-10. Using the mouse MuMig cDNA probe, HuMig a new human member of the family was discovered. Our mapping studies have placed the human mig and IP-10 genes to within 14 kilobases of each other on the long arm of chromosome 4 at a site distant from the cluster of other chemokine genes.</p> <p>Work to date has demonstrated that the MuMig and HuMig mRNAs accumulate in monocytic cells specifically in response to gamma interferon, while Crg-2/IP-10 can respond to alpha interferon and to lipopolysaccharide as well.</p> <p>By PAGE, the Mig proteins show multiple species with mobilities corresponding to 16-20 kDa for MuMig and 9.5-14.3 kDa for HuMig. HuMig was purified from transfected CHO cells. N-terminal sequencing and mass spectrometry have revealed that HuMig's heterogeneity is due to carboxy-terminal truncations.</p> <p>Functional studies have revealed that HuMig targets activated T cells, causing a rise in intracellular calcium and chemotaxis. Resting T cells, neutrophils, monocytes, and EBV-transformed B cells do not respond to HuMig.</p> <p>Ongoing work is concentrating on determining the range of biological effects of Mig on T cells; on identification of Mig receptors and other novel chemokine receptors on lymphocytes; and on identifying additional novel chemokines.</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00687-03 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Structure-Function Analysis of the Immediate-Early Proteins of alphaherpesviruses**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

**Principal Investigator:** L. P. Perera, DVM; Ph.D., Senior Staff Fellow

**Other:** S. E. Straus, MD., Senior Investigator, LCI, NIAID

M.S. Nakamura, MD, Medical Staff Fellow, LCI, NIAID

C. R. Brown, MS, Senior Laboratory Technician, LCI, NIAID

COOPERATING UNITS (if any)

**Collaborators:** G. S. Hayward, Ph.D., Johns Hopkins University, Baltimore, MD; J. D. Mosca, Ph. D., Henry M. Jackson Foundation, Rockville, MD.

LAB/BRANCH

LCI

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.2

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Herpes simplex virus (HSV) and Varicella-Zoster virus (VZV) are alphaherpesviruses that cause oral/genital herpes and chickenpox/zoster respectively. They are enveloped viruses with relatively large genomes with the coding capacity for more than 70 unique genes. The expression of genes in alphaherpesviruses are temporally regulated and three putative kinetic classes have been defined: immediate early, early and late genes. The immediate early genes encode proteins that regulate the expression of genes of other kinetic classes thus playing a pivotal role in the life cycle of the herpesviruses. These alphaherpes viral immediate early proteins not only provide excellent model systems to study eukaryotic gene regulation in general but also understanding the regulatory mechanisms of these proteins provide potential molecular targets that could be exploited for therapeutic interventions. Current efforts focus on four HSV regulatory proteins: ICPO, ICP4, ICP27 and VP16. Our recent studies indicate that the ICP27 is a RNA-binding protein that stabilizes unstable mRNA by specifically interacting with the 3' RNA-processing signals of a particular transcript. In addition, the ability of this protein to interact with the basal transcription factors TFIIB and TBP has been demonstrated. To identify the cellular proteins that interact with these HSV regulatory proteins to mediate their transcriptional effects, we have made use of the "Yeast two-hybrid" system and are currently screening a cellular cDNA library derived from HeLa cells. The three VZV transregulatory proteins under investigation are IE62, ORF4 and ORF10. Our recent studies have illustrated that the IE62-dependent activation of responsive promoters are mediated by a unique mechanism involving the TATA element of the promoter. Studies are in progress to delineate the interactions of IE62 with the transcriptional machinery of the Pol II promoters and the preliminary evidence indicate that IE62 not only interacts with the basal transcription factors TFIIB and TBP but also interacts with the cellular transcription factor USF. Using, saturation and site specific mutagenesis, further dissection of the functional domains of IE62 are being pursued.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00688-03 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of herpesvirus interactions with cell surface molecules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principle Investigator: Richard K. Williams - Senior Staff Fellow LCI/NIAID

Others: H. Kimura - Visiting Fellow LCI/NIAID

M. Moriuchi - Special Volunteer LCI/NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanisms of binding and entry of varicella zoster virus (VZV) into cells are unknown. We are investigating the binding of VZV to cell membrane preparations from a variety of cultured cell-lines, to isolated proteins, glycoconjugates and to synthetic peptides. Inhibition of VZV infectivity in diploid human lung cells is being used to assess potential receptor-mimicking molecules for ability to block VZV binding and infection of cells. Monoclonal and polyclonal antibodies to cell membrane proteins are being developed to identify the specific cell surface molecules that serve as receptors for VZV. We found that heparin inhibited infectivity of VZV, in contrast another glycosaminoglycan, chondroitin sulfate did not inhibit VZV infectivity. VZV binding to cell membrane preparations was inhibited by added heparin suggesting that an important mechanism of interaction of VZV with cells involves heparin. Several assays were developed to measure binding of VZV and of herpesvirus glycoproteins to immobilized heparin. VZV was found to bind to immobilized heparin. Soluble VZV gB (gpII) has been produced using the vaccinia-T7 expression system to investigate glycoprotein to heparin receptor binding using ELISA based and biosensor technologies. The interaction of glycoprotein B of herpes simplex virus type 2 (HSV-2) with heparin-like glycosaminoglycans is being investigated using the biosensor technique. This study will lead to a detailed understanding of the affinity and specificity of the HSV gB to heparin interaction and serves as a model system to explore inhibitor compounds that may be used to interrupt the interaction of herpesviruses with their cellular receptors and therefore act as antiviral drugs.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00704-2 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Cytokine production in animals infected with Histoplasma Capsulatum

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert Seder, M.D. Investigator LCI/NIAID  
Others: Ping Zhou, M.D. Fogarty Fellow LCI/NIAID

COOPERATING UNITS (if any)

Mycology Section, LCI

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Lymphokine Regulation Unit

INSTITUTE AND LOCATION

NIAID, National Institute of Allergy and Infectious Disease, Bethesda, Maryland

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to understand cytokine regulation in animals infected with Histoplasma Capsulatum. Histoplasmosis is a disease found in certain geographic regions of this country. In a normal host the disease will often be controlled without significant end organ damage. However, patients infected with HIV have a difficulty in eradicating the disease. Therefore it was of interest to study mechanisms which might alter the immune response to this organism with the goal of protecting the animal. Previous work had demonstrated that the organism resides in mononuclear phagocytic cells. Moreover, it was shown that the presence of IFN $\gamma$  enables the macrophage to kill the organism. Recently, a new cytokine, IL-12 has been shown to be a potent inducer of IFN $\gamma$  from both T cells and NK cells. Thus we set out to investigate the role that IL-12 had in the course of the disease and how it affected cytokine production especially IFN $\gamma$ . Mice infected with *H. capsulatum* and treated with neutralizing antibodies to IFN $\gamma$ , TNF $\alpha$  or IL-12 had accelerated mortality, indicating that endogenous production of these cytokines plays an important role in response to infection. In contrast, mice treated with IL-12 or a neutralizing antibody to IL-4 at the initiation of infection had substantially diminished mortality. Moreover, mice infected and treated with IL-12 show a 2-3 fold increase in the amount of IFN $\gamma$  following in vitro stimulation with specific *H. capsulatum* antigen compared to the control infected mice. The protective effect of IL-12 could be abrogated if a neutralizing antibody to IFN $\gamma$  was given at the same time, demonstrating that the role of IL-12 in protection was mediated by IFN $\gamma$ .



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00705-2 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Cytokine Production from patients infected with HIV and TB.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert A. Seder, M.D., Lymphokine Regulation Unit, LCI, NIAID  
Others: John McDyer, M.D., Medical Staff Fellow, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Lymphokine Regulation Unit

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Disease

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We observed that IL-15 enhances the proliferative response in a dose dependent manner from PBMCs of HIV infected individuals when stimulated by polyclonal mitogen, tetanus toxoid, or HIV specific antigen. The effects of exogenous IL-15 are substantially diminished by adding a neutralizing antibody to the  $\beta$  chain of the IL-2 receptor. Moreover, the ability of IL-15 to increase proliferation is enhanced by the presence of endogenous IL-2 produced in the cultures. Addition of IL-2 or IL-15 to short term in vitro cultures of either PBMCs or CD4+ T cells had little effect on IL-2, IL-4 or IFN $\gamma$  production. By contrast, IL-12 caused substantial enhancement of both IL-2 and IFN $\gamma$  production from these cultures. The role that endogenous cytokines have on IFN $\gamma$  induction was also studied. Addition of neutralizing antibody to the  $\alpha$  chain of the IL-2 receptor or IL-12 to antigen stimulated cultures caused a striking decrease in IFN $\gamma$  production. Neutralization of endogenous IL-15 also resulted in diminished IFN $\gamma$  production from cultures stimulated with mitogen. IL-4 and IFN $\gamma$  protein production by PBMCs and CD4+ T cells stimulated with mitogen was assessed to see if we could detect a specific bias of cytokine production. Small amounts of IL-4 were detected from CD4+ T cells but not PBMCs from most individuals tested. However, IFN $\gamma$  and IL-2 were also produced from these same cultures.

The development of multi-drug resistant Tuberculosis has developed into a major health problem. Using the same techniques as outlined above, we will examine the functional capabilities of PBMC's and CD4+ T cells from patients infected with Tuberculosis.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00708-02 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Clinical Basis of Food Allergy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Allergic Diseases Section, LCI/NIAID

COOPERATING UNITS (if any)

Indian Institute of Science, Bangalore, India (P. V. Subba Rao)  
Clinical Neuroscience Branch, NIMH (Brian Martin)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.20

PROFESSIONAL:

0.10

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The major shrimp allergen is now known to be shrimp tropomyosin. Antigenic as well as allergenic activities are associated with at least two peptides that are similar between Crustaceae, but vary between tropomyosins from phylogenetically distant species. Synthetic peptides corresponding to IgE binding B-cell epitopes have comparable allergenicity to native peptides. T-cell epitopes are now being identified.

Pollen of *Parthenium hysterophorus* is a major cause of allergic rhinitis throughout the world. We have now purified a 31 kDa acidic glycoprotein, rich in glycine and proline, from *Parthenium hysterophorus* (designated as Par h 1). Pronase digestion, periodate oxidation and chemical deglycosylation experiments strongly suggest that the oligosaccharides covalently linked to the polypeptide backbone of Par h 1 contribute significantly towards its binding to IgE antibodies. The N-terminal sequence shows a strong identity with an anther-specific cell wall protein. The hydroxyproline-rich region of Par h 1 also exhibits partial identity in a 30-40 amino acid sequence to extensins, a class of hydroxyproline-rich cell wall glycoproteins from maize, tobacco, *Sorghum* and carrot. Antibodies to Par h 1 cross-react with an extensin precursor from potato tuber. This data is consistent with the conclusion that, like crustacean tropomyosins and plant profilins, a group of soluble plant glycoproteins related to the ubiquitous extensins have certain common IgE binding epitopes that contribute to allergenic cross-reactivity among specific pollens and foods.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

201 AI 00709-02 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lymphokine Profiles in Asthma and Allergic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Allergic Diseases Section, LCI/NIAID  
 Others: Calman Prussin, M.D., Medical Staff Fellow, LCI/NIAID  
 Vanitcha Rumsaeng, M.D., Visiting Associate, LCI/NIAID

COOPERATING UNITS (if any)

Laboratory of Parasitic Diseases (Dr. Thomas Nutman)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.50

PROFESSIONAL:

1.20

OTHER:

0.30

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recently, there have been several reports demonstrating improvements in the flow cytometric detection of intracellular cytokines. These advances, although significant, have not yielded techniques that have easily been translated into broad use. To address this issue, we have coupled a fixation and permeabilization method with the use of directly labelled monoclonal anti-cytokine antibodies, providing both improved signal and simpler staining. The specificity of this technique can be demonstrated by blocking cytokine staining using a molar excess of recombinant cytokine. Additionally, unlabelled anti-cytokine antibodies block specific staining of labelled antibody, providing an objective means to place statistical markers. Using such controls, we routinely detect as few as 0.1% false positive cells, allowing the flow cytometric detection of IL-5. This technique was used to detect intracellular cytokines. We examined peripheral blood T cell subpopulations to determine the cytokines produced by individual lymphocytes. Cells from normal individuals and helminth infected patients were sorted for the CD4+CD27+ and CD4+CD27- subpopulations. Intracellular staining for IL-4, IL-5, and IFN- $\gamma$  revealed that virtually no CD4+CD27- lymphocytes produce both IL-5 and IFN- $\gamma$  while a distinct proportion produced both IL-4 and IFN- $\gamma$  (0.1-8.0%) and 66-84% of IL-5-producing cells also produce IL-4. Lymphocytes from normals and patients had the same functional T cell subsets, but the CD4+CD27- lymphocytes from patients had higher frequencies of cells producing IL-4 or IL-5. Normal subjects had higher frequencies of cells producing IFN- $\gamma$ . We are now applying this technique to the characterization of cells entering the airways after segmental challenge with antigen. Initial results suggest that the lymphocytes that initially enter the airways are identical to the circulating lymphocyte population in their cytokine profile.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00710-02 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Homologs of Herpesvirus Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J. Cohen, Senior Investigator, LCI, NIAID  
 S. Mallipeddi, IRTA Fellow, LCI, NIAID

COOPERATING UNITS (if any)

M. Spriggs, Immunex Corp., Seattle, Washington

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1) Herpesviruses cause latent infections that persist for the lifetime of the host and these viruses have developed mechanisms to counteract host defenses so as to allow the virus to persist. Using sophisticated computer programs we are identifying herpesvirus genes that may have cellular homologs. These genes are being expressed in various systems to determine how the genes may interact with host cell proteins, including those of the immune system, to influence the course of infection. Identification of these genes may help define new targets for antiviral therapy or new insights into modulating the immune system. 2) Epstein-Barr virus (EBV) encodes a type II membrane protein, BZLF2, that is homologous to members of the C-type lectin family. In collaboration with Immunex Corporation, we have constructed a soluble fusion protein containing the human IgG Fc domain linked to the extracellular domain of BZLF2. The extracellular domain of BZLF2 was found to bind to the human MHC class II HLA-DR  $\beta$  chain. Site directed mutagenesis of the HLA-DR  $\beta$  chain indicated that the  $\beta$ 1 domain was required for recognition of the extracellular domain of BZLF2. Since this domain participates in forming the peptide binding pocket, these results suggested that BZLF2 may interfere with class II directed antigen presentation. The fusion protein containing the extracellular domain of BZLF2 inhibited antigen presentation and generation of antigen-specific cytotoxic T lymphocytes in mixed lymphocyte cultures. Current studies are directed toward inactivating the BZLF2 gene in EBV to determine the role of the protein during growth of the virus both in cell culture and in an animal model. 3) Varicella-zoster virus (VZV) ORF13 encodes the viral thymidylate synthetase. This protein has 68% amino acid identity to the human thymidylate synthetase. We have constructed a mutant in the Oka vaccine strain of VZV that cannot express the viral thymidylate synthetase by inserting stop codons into the gene. We plan to inoculate guinea pigs with the VZV mutant to determine whether viral thymidylate synthetase is important for infection with the virus or for the ability to spread to the central nervous system and maintain latent infection. 4) Additional herpesvirus homologs of known mammalian proteins have been identified. Promising candidates will be studied in appropriate systems in collaboration with M. Spriggs at Immunex Corporation.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00726-02-LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies on *Candida* Species

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K.J. Kwon-Chung, Ph.D. Research Microbiologist, Clinical Mycology  
Section, LCI/DIR/NIAID  
Others: Ram Petter, Ph.D. Fogarty Fellow, Clinical Mycology Section  
LCI/DIR/NIAID

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID/NIH - Bethesda, Maryland

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

*Candida albicans* is the most frequently isolated fungal pathogen in humans. It occurs in two distinct biotypes differentiated by their ability to assimilate sucrose. The strains unable to assimilate sucrose have been identified at *C. albicans*, var. *stellatoidea*. The *stellatoidea* variety was first isolated from vaginal mucosa in the 1940's in the United States but was rarely found among the isolates obtained from immunocompetent individuals. Since 1980, increasingly frequent isolations of the sucrose negative variety has been reported from HIV positive patients. Our previous studies indicated that the *stellatoidea* variety not only differs from the type variety in its ability to assimilate sucrose but also in its karyotype, linkage map and virulence in the animal model. When the sucrose negative phenotype is reverted to sucrose positive, it also gains the ability to assimilate glycerol, methyl-D-glucoside and maltose, and has increased virulence in the animal model. These phenomenon suggest a mutation in the regulatory gene(s) and resembles the phenotypic characteristics of *Saccharomyces snf1* mutants. We have chosen to study *SNF1* genes in two biotypes of *C. albicans*. *SNF1* gene encodes a serine-theonine protein kinase which was first identified as a gene essential for sucrose utilization in *Saccharomyces cerevisiae*. Further studies suggested the *SNF1* is responsible for derepression of the *SUC2* gene expression which is necessary for the assimilation of sucrose and also plays a role in the regulation of lipid metabolism, cell cycle, sporulation and most likely other important mechanisms in the two biotypes of *C. albicans*. Using the PCR method, *Candida SNF1* gene was isolated and characterized. *Candida* gene had high similarity at the amino acid level with that of *Saccharomyces* and was able to complement the *snf1* phenotype of *Saccharomyces*. The *SNF1* gene was assigned to chromosome # 5 in *C. albicans* and to two different chromosomes in the *Stellatoidea* variety. To investigate the role of *SNF1* in *Candida*, disruption of the two homologs was attempted using the *URA3-hisG* cassettes which had been successfully applied for deletions of other genes in *C. albicans*. We disrupted one homolog and found that the *snf1* mutation is recessive as is the case in *Saccharomyces*. The results up to this point indicate that the *SNF1* gene is highly conserved in fungi and that the two biotypes of *C. albicans* are different in the *SNF1* gene at the chromosomal level.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00732-01 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical/Immunologic/Genetic Analyses of Autoimmune Lymphoproliferative Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Stephen E. Straus, M.D., Chief, LCI, NIAID  
 Others: Janet K. Dale, R.N. Research Nurse, LCI, NIAID  
 Warren Strober, M.D. Head, MIS, LCI, NIAID  
 Michael Lenardo, M.D. Senior Investigator, LI, NIAID  
 Michael Sneller Head, IDS, LIR, NIAID

COOPERATING UNITS (if any)

Jennifer Puck, M.D. NCHGR  
 Albert Lin, M.D. NCI

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.4

PROFESSIONAL:

0.4

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1990 we identified a child with a disorder involving dramatic enlargement of all her lymph node chains, hepatomegaly, splenomegaly, autoimmune hemolytic anemia and marked expansion of CD3+/CD4-/CD8- T cells. We have termed this disorder autoimmune lymphoproliferative syndrome, or ALPS. We subsequently identified eight additional children with similar disorders; their clinical and immunologic features were well-studied. During the past year, we discovered heritable, functional mutations—in the first 5 cases tested—in the gene encoding Fas, a cell surface protein involved in lymphocyte apoptosis. This is the first genetic disorder involving apoptosis and the first human gene in which a defect leads to autoimmune disease. We will be analyzing other proteins related to apoptosis to determine whether they contribute to ALPS.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00725-01 LCI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Activities of Chemokines In Vivo

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joshua M. Farber, Investigator, LCI/NIAID

Others: Doron Amichay, Visiting Fellow, LCI/NIAID

COOPERATING UNITS (if any)

Alan Sher, LPD, NIAID; Ricardo Gazzinelli, LPD, NIAID; Gunasegaran Karupiah, LIP, NIAID; Giovanna Tosato, CBER, FDA; Stephen Shaw, EIB, NCI; Heiner Westphal, NICHD

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Cytokine Biology Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The goal of the project is to investigate the biological roles of members of the chemokine family of cytokines by studying chemokine actions *in vivo*, primarily in animal models of infectious disease.

Through differential screening of a cDNA library prepared from lymphokine-activated macrophages, this laboratory discovered two mouse chemokines, Crg-2 and Mig. Crg-2 is likely the mouse homologue of the human chemokine IP-10, and the murine Mig (MuMig) was used to identify a human homologue, HuMig. Crg-2/IP-10 and Mig are related, interferon-gamma inducible chemokines that preferentially target T lymphocytes, acting as chemotactic factors for activated T cells.

Following injection of mice with interferon-gamma, *mig* was induced dramatically in multiple organs. In analyses of animal models infectious disease, infection of mice with the malarial parasite *P. Yoellii* induced *mig* in liver, spleen, lung and heart. Infection of mice with the protozoa *T. gondii* led to induction of *mig* early after infection in liver, lung, spleen and heart and at later times in brain. Infection of mice with vaccinia virus induced *mig* in liver, ovary, uterus, spleen, heart and lung. Using interferon-gamma knockout mice or anti-interferon-gamma antibodies, we demonstrated that inductions of *mig* by infection with *T. gondii* and vaccinia virus were dependent absolutely on interferon gamma. Expression of Crg-2/IP-10 of these models was similar to that of *mig*, but with notable exceptions. We presume that Mig and Crg-2/IP-10 are mediating interferon-gamma dependent effects on the trafficking of activated T cells and that Mig's and Crg-2/IP-10's activities are important for host defense against a variety of pathogens. Ongoing work is concentrating on more precise localization of expression of *mig* and other chemokines in tissues and cells in the animal systems and on experiments to determine the biological roles for the chemokines in these systems, including the production of mice with targeted deletion of the *mig* gene.





LABORATORY OF HOST DEFENSES  
1995 Annual Report  
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PHS-NIH

Summary Statement

LABORATORY OF HOST DEFENSES  
National Institute of Allergy and Infectious Diseases

October 1, 1994 to September 30, 1995

**Introduction**

The objective of the LHD is to integrate basic and clinical research of the role of phagocytic cells in host defense and inflammation. The focus of work is (1) to study the physiology, biochemistry and molecular biology of phagocytic cells (blood neutrophils, mononuclear phagocytes, eosinophils) in host defense against bacteria and fungi; (2) to determine the role and effect of cytokines on both phagocytic cells and upon the general process of inflammation; (3) to investigate the cellular, biochemical and genetic basis of diseases leading to recurrent bacterial infections, particularly diseases affecting phagocytic cell function (chronic granulomatous diseases of childhood (CGD), Hyperimmunoglobulin E-recurrent infection syndrome, neutrophil specific granule deficiency, leukocyte adhesion deficiency, cyclic neutropenia and others); (4) to develop new animal models of human diseases which lead to recurrent infections; (5) to develop new treatments for host defense defects leading to recurrent infections including new antibiotic or cytokine therapy or prophylaxis against infection, with a particular emphasis on tuberculosis and atypical mycobacterial infections; and (6) to develop gene therapy for the inherited defects of phagocyte function, particularly chronic granulomatous diseases. In addition to these studies the LHD serves as a national resource for assisting physicians throughout the country (and the world) in the evaluation of patients with host defense defects.

During the past year the Laboratory has continued and expanded upon clinical studies in patient cohorts collected at NIH over more than 20 years. Studies have been performed in patients and normal volunteers regarding the physiology and biochemistry of phagocytic cells and cytokines in infection and inflammation. The oxidative microbicidal superoxide-generating NADPH oxidase, the microbicidal granule proteins, and the chemoattractant receptors of phagocytic cells were studied. Also during the past year important mouse models of human disease were established including a knockout model of chronic granulomatous disease, and an informative model for low dose radiation enhanced autologous blood progenitor transplantation.

While detailed descriptions of individual projects are included in the individual project reports, the following is a list of some of the major accomplishments of the laboratory:

- o Initiation of the first clinical trial of retrovirus mediated gene therapy for chronic granulomatous disease; development of a system of blood stem cell targeted gene



therapy incorporating the enhanced safety features of a sterile closed gas permeable plastic bag system of culture and the use of culture medium free of animal proteins.

- o Five-year follow up of CGD patients receiving interferon  $\gamma$  reveals continued efficacy and no serious adverse side effects.

- o Demonstration that mononuclear cells from patients with a familial X-linked form of disseminated infection with multiple drug-resistant (MDR) atypical mycobacterial have diminished production of both interleukin 12 and interferon- $\gamma$ .

- o Demonstration that interferon- $\gamma$  has important therapeutic potential for disseminated infection with MDR atypical mycobacterial infection and could also be useful in the treatment of multiple drug resistant tuberculosis.

- o Characterization of the orderly recruitment of cytokines into skin blisters of humans, indicating an early and extensive production of IL8.

- o Demonstration that exudate neutrophils are induced to synthesize and release IL-8, and that this effect can be stimulated in circulating neutrophils by agents that increase intracellular ionized calcium.

- o Characterization of the appearance of cytokines and demonstration of elevated E-selectin in the circulation following endotoxin administration to normal human volunteers, together with demonstration that exudate neutrophils have a marked depletion of surface L-selectin.

- o In vitro correction of stem cells from patients with each of the four types of chronic granulomatous disease (CGD) using gene transfer technology.

- o Demonstration that recruitment of CD34+ hematopoietic stem cells to the peripheral blood by administration of granulocyte colony stimulating factor (G-CSF) follows distinctly different kinetics than the blood neutrophil counts, peaking on day 5 and 6 and declining thereafter; Demonstration that G-CSF recruitment of CD34+ cells, but not neutrophils is significantly reduced in patients with CGD and in another group of patients with SCID adenine deaminase deficiency.

- o Use of a mouse marrow transplant model based on the congenic C57/black Ly5.1 and 5.2 strains to demonstrate that cytokine conditioning and low dose radiation can facilitate transplant efficiency leading to significant chimerism; a result with important implications for enhancing efficiency of engraftment of autologous gene therapy corrected blood progenitors.

- o Establishment of a colony of gene "knockout" mice with p47phox deficient chronic granulomatous disease, which model the human form of the this disease including



spontaneous severe infections with bacteria and fungi; the first demonstration in an animal model of CGD that interferon gamma administration reduces frequency of severe infections.

- o Demonstration that src homology 3 (SH3) domains are required for the assembly of NADPH oxidase components. Demonstration of proline rich sequences as targets for SH3 binding during NADPH oxidase assembly; demonstration that the mutation of one proline in p22phox accounting for one of the CGD genotypes also impairs SH3 binding by p47phox.

- o Cloning and sequencing of human and mouse clusters of cross-hybridizing genes that encode heptahelical, rhodopsin-like, G-protein coupled phagocyte chemoattractant receptors or related products of unknown function; sequence relationships indicate these genes arose from a common ancestor. Of note is the cloning of the human MIP1/rantes receptor and the establishment of a knockout mouse devoid of this receptor.

- o Demonstration that during primate evolution eosinophil cationic protein and eosinophil-derived neurotoxin (small granule proteins that are both members of the ribonuclease gene family) accumulated non-silent mutations at rates that exceed those of all other functional genes studied in primates, while retaining the structural features essential for promoting ribonuclease activity.

### **Clinical Studies**

Over the past three years, studies have been in progress to develop methods of gene transfer of normal genes for the phagocyte NADPH oxidase into hematopoietic progenitor cells of CGD patients defective in such genes. These studies have culminated in the initiation of a Phase I trial of gene therapy for the p47phox autosomal recessive form of CGD. The first patient has been treated and the followup is in progress. Plans are to treat four additional patients before designing the next phase of these studies. The tools to extend these studies to the other three genetic forms of CGD have been developed.

A LHD study completed four years ago demonstrated the efficacy of prophylactic interferon-gamma to reduce infection frequency in patients with chronic granulomatous disease (CGD). We have entered the fourth year of a phase IV study aimed at determining the long term safety for the patients and the continued effect of interferon- $\gamma$  on both phagocytic cells and the inflammatory process. This treatment appears to be without adverse effects and patients continue to benefit from interferon- $\gamma$ . A double-blinded randomized trial was continued to determine whether prophylactic oral itraconazole can reduce fungal infections in CGD. In related studies on the patient group with CGD, data were collated and published relating to infections with unusual fungal organisms.

Studies of patients with Mycobacterium infections have continued with patients infected with mycobacterium avium intracellulare (MAI), but have been expanded to also include patients with multiple drug resistant M. tuberculosis. A family group with X-linked susceptibility to





MAI was further characterized indicating a defect in monocytes affecting interleukin 12 production in addition to the previously defined impairment of monocyte mediated lymphocyte interferon gamma production. Studies to date demonstrate that interferon gamma is well tolerated in these patient groups and may provide benefit together with antibiotic therapy in controlling infection.

Clinical studies of normal volunteers and patients were continued to determine the systemic and local changes in cytokines and phagocytic cells in response to both local inflammation, modelled by experimental skin blisters and systemic responses modeled by reaction to intravenous bacterial endotoxin. Recent studies of patients with abnormal inflammatory responses revealed dramatic increases in TNF- $\alpha$  in patients with acute vasculitis in association with Wegeners granulomatosis, in patients with systemic mastocytosis, and in the syndrome of hyperimmunoglobulin E and recurrent infections, with increased TNF- $\alpha$  correlating best with disease activity. Skin blister studies indicated a correlation to the clinical data with patients with vasculitis showing markedly elevated skin blister TNF- $\alpha$  levels with active disease and normalization of TNF- $\alpha$  with remission. These observations have prompted a clinical trial using a drug that blocks TNF- $\alpha$  synthesis (lysophylline). In other related studies normal blister exudate neutrophils were shown to synthesize greatly enhanced amounts of IL-8 compared with control peripheral blood neutrophils. Studies of normals and patients with excessive inflammation revealed a tight linear relationship between the level of IL-8 and the number of inflammatory neutrophils in the exudate. This increased production/release of IL-8 could be reproduced by increasing intracellular calcium in peripheral blood neutrophils. In related studies exudative neutrophils were shown to have shed one of the important adhesion molecules, L-selectin. This is an important distinction between peripheral blood and exudative cells.

A clinical study was initiated to look at the recruitment of CD34 peripheral blood progenitors from the marrow in response to administration of G-CSF. Results of this study indicate that an optimum dose is 10 ug/kg/day administered subcutaneously for 5 or 6 days. In most individuals this results in an increase in circulating CD34 cells from 1000 cells/ml whole blood to over 40,000 CD34 cells/ml whole blood. The kinetics of recruitment are distinctly different from neutrophils. Neutrophils increase by 6 hours of the first dose of G-CSF increasing rapidly over the first 24 hours after administration, and then increase a small amount further with each day of administration. CD34+ cells do not begin to increase until day 3 reaching a peak at day 5 or 6 and then steadily decline after that. In certain patient groups that would be the target of gene therapy (chronic granulomatous disease and adenine deaminase deficiency) there is significantly decreased recruitment of CD34+ cells. This information is essential to the planning of harvest of progenitors for clinical treatments such as gene therapy requiring autologous transplantation of gene altered CD34+ cells.

#### **Basic science studies of phagocytic cells**

Studies of the growth of peripheral blood progenitor cells in culture are essential to development of gene therapies based on the introduction of new genes into these cells.



Conditions have been defined to optimized growth toward the neutrophil, monocyte or eosinophil lineage. Specifically, the combination of stem cell factor, Interleukins-3 and 6, GM-CSF, and G-CSF is optimal for neutrophil differentiation and SCF, IL-3 and GM-CSF for monocyte/eosinophil production. The combination of IL-3, GM-CSF and IL-5 result in almost exclusively eosinophil development from CD34 cell hematopoietic progenitors.

In our laboratory all of the protein components of the phagocyte superoxide-generating NADPH oxidase have been identified. The next phase of studies has been focussed on the molecular domains that mediate interaction between oxidase components. Specifically the src-homology 3 domains (SH3) of the p47phox and p67phox proteins appear to bind to target proline rich domains of the p22phox and the p47phox respectively. In related studies the SH3 domains were shown to be absolutely essential for oxidase activation in the intact cell, but may not be essential in a cell-free activation of the oxidase, suggesting the importance of these domains for the complex events occurring in the intact cell.

A CGD knockout mouse model for the p47phox autosomal recessive form of CGD has been established. This model reproduces the human disease resulting in severe bacterial and fungal infections associated with granuloma formation. Already this model has been used to confirm the use of interferon gamma as effective prophylaxis against infection in CGD. This model will be extremely useful to develop new treatments for CGD and to understand the physiologic role of oxidants in pathogenesis of inflammation.

Studies of the molecular evolution of eosinophil granule proteins in primates has revealed accelerated evolution. This builds on other studies in the LHD which demonstrated the accelerated evolution of host defense proteins relative to other proteins in a mouse/human comparison.

The 1° structure, expression and signaling properties have been established for the human N-formyl peptide receptor (FPR), the FPRL1 receptor (FPRL= formyl peptide receptor like), interleukin-8 receptor type B (IL8RB), and the MIP-1 $\alpha$ /RANTES receptor from cDNA and gene cloning. Similar work has been done for the mouse leading to the establishment of a knockout of the MIP1  $\alpha$ /RANTES receptor. Work is in progress characterizing the phenotype of this mouse disease model. In other studies it was found that open reading frame *ECRF3* of *Herpesvirus saimiri* encodes a protein with 33% amino acid sequence identity to IL8RB that is a functional receptor for the same ligands that activate IL8RB (IL-8, GRO/MGSA and NAP-2). The MIP-1 $\alpha$ /RANTES receptor also has a viral homologue, *US28* of human cytomegalovirus. These findings contribute to an emerging theme in DNA virology whereby molecular mimicry of host defense proteins is accomplished by gene copying.

#### **Administrative, Organization, and Other Changes**

Dr. John I. Gallin continued as the Chief and Dr. Harry L. Malech as Deputy Chief of LHD. Dr. Murphy continues as a tenured scientist. Dr. Thomas Leto was considered by the NIAID tenure committee and received a recommendation for his application to be forwarded to the



central NIH tenure committee. Dr. Helene Rosenberg and Dr. Steve Holland continue as tenure track scientists in the Laboratory.

### **Honors, Awards, and Scientific Recognition**

Dr. John I. Gallin was appointed Director of the Warren Grant Magnuson Clinical Center, NIH and Associate Director of Clinical Research for NIH on May 1, 1994, . Dr. Gallin was asked to serve on the Editorial Board of the Journal of Biological Chemistry starting January 1996 and on the Advisory Board of Cellular Immunology. In addition he was appointed to the Scientific Advisory Committee for the Immune Deficiency Foundation's Chronic Granulomatous Disease Registry. He is the immediate past President of the International Immunocompromised Host Society. In 1994-1995 Dr. Gallin was invited to deliver numerous lectures and grand rounds. In 1995 he was promoted to the rank of Rear Admiral (upper) in the U.S Public Health Service Commissioned Corps.

Dr. Harry L. Malech, as principal investigator of a Phase I clinical trial of gene therapy for chronic granulomatous disease, completed all regulatory approvals for this trial during FY '95 and has enrolled the first patient in this protocol in July 1995. During FY 1995, Dr. Malech continues as a member of the Editorial Board of the publication Immune Deficiency and Allied Disorders: Clinical Updates. Dr. Malech continues as a member of the NIH Patent Policy Board, Subcommittee on Cooperative Research and Development Agreements. He also was appointed to the NIH Museum committee planning the genetics exhibit for NIH. Dr. Malech continues as a member of the Georgetown University Department of Medicine Chairman's Advisory Committee. Dr. Malech continues to receive invitations to chair and speak at scientific meetings including conferences in Japan, Australia, Florida, New York, and Washington. Dr. Malech also presented to the NIH Staff Training in Extramural Programs (STEP) lecture series a talk entitled "Human Gene Therapy" on March 23, 1995.

Dr. Philip Murphy in FY '95 was appointed Section Editor, *The Journal of Leukocyte Biology*.

He was also appointed as a Consultant to Pharmacoepia, Inc. In 1995 he was the inventor on a patent issued to the NIH for cloning of a cDNA for human interleukin-8 receptor type B.

Dr. Thomas Leto in FY95 was an invited lecturer at a number of scientific meetings. He was also presented to the NIAID tenure committee for consideration of tenure appointment, with a recommendation to forward his tenure package to the NIH central tenure committee.

Dr. Steven Holland in FY '95 created a mouse knockout model of the p47phox deficient form of the human disease, chronic granulomatous disease. Dr. Holland was appointed as the NIAID representative to the Reinvention of Government (REGO) committee for NIH. He continues to be invited as a speaker at scientific meetings.

Dr. Helene Rosenberg in FY '95 has published studies outlining the molecular evolution of eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN), two important



host defense proteins with RNase activity. She demonstrated that the genes encoding EDN and ECP have accumulated non-silent mutations at rates that exceed those of all other functional genes studied in primates, while retaining the structural features essential for promoting ribonuclease (RNA-destroying) activity.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00155-20 LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Abnormal Host Defense

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: John I. Gallin, M.D. Laboratory Chief LHD/NIAID

Others: Harry L. Malech, M.D. Deputy Laboratory Chief LHD/NIAID  
 Steven M. Holland, M.D. Senior Staff Fellow LHD/NIAID  
 Ellen DeCarlo Research Nurse LHD/NIAID  
 Judi Miller Research Nurse LHD/NIAID

COOPERATING UNITS (if any)

D Kuhns, Ph.D., PRI-Frederick Cancer Research Facility; Julia Lekstrom, JE Bennett, & KJ Kwon-Chung, LCI/NIAID; HI Pass, Surgery Branch/NCI; M Sneller, LIR/NIAID; Genentech

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study patients with abnormal host defense. In FY'95 studies focussed on patients with abnormal phagocyte function. These included patients with chronic granulomatous disease of childhood (CGD), Hyperimmunoglobulin E-recurrent infection syndrome (Job's), leukocyte adhesion deficiency (LHD) and other patients with recurrent infections who do not fall into a specifically defined disease category. Currently we follow over 80 patients with CGD, 18 patients with the hyperimmunoglobulin-E recurrent infection syndrome, and over 20 patients with other phagocyte dysfunction syndromes. In addition, 12 patients are followed with disseminated multiple drug resistant-atypical mycobacteria infections, some of whom appear to have abnormalities of phagocytic cells. A phase IV study of the effect of interferon-γ for infection prophylaxis in CGD has continued at NIH at the specific request of the FDA. No unexpected toxicities have been seen with interferon-γ. The reduction in infections observed in the initial study has been sustained and of note is that the incidence of fungal infections in CGD patients receiving interferon-γ is half that for those patients not receiving the drug. In view of the high incidence of fungal infections in CGD patients, we have also initiated a study designed to assess the efficacy of itraconazole in CGD. Several long term studies were completed related to specific management issues of CGD patients. These included the surgical management of pulmonary infections in CGD, and the surgical pathology of the lung in CGD. In addition we described suppurative cutaneous granulomata caused by Microascus cinereus in a patient with CGD as well as the successful treatment of severe liver abscesses with local installation of neutrophil enriched leukocytes into liver abscess. Each of these clinical studies could only be compiled at NIH where a large cohort of CGD patients could be followed for a long time. Several unique infections and their successful management were described in CGD. These include reports of successful treatment of Sarcinosporon inkin, Exophiala and other fungi.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00481-09 LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Phagocyte NADPH Oxidase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Daniel Rotrosen, MD Medical Officer LHD/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project Terminated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00521-08 LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Cytokines in Host Defense and Inflammation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John I. Gallin, M.D. Laboratory Chief LHD/NIAID

Others: Michael Sneller, M.D. Staff Physician LIR/NIAID  
Ellen DeCarlo Research Nurse LHD/NIAID

COOPERATING UNITS (if any)

Douglas Kuhns, Ph.D., PRI-Frederick Cancer Research Facility

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies were performed to define the accumulation of cytokines and other mediators of inflammation in experimental models of inflammation in normal human subjects and in patients with abnormalities of host defenses or inflammation. Skin blisters created by suction and heat were used to study local inflammation in the skin. This blister system has been used by us to characterize the accumulation of inflammatory mediators. Studies of skin blisters in patients with abnormal inflammatory responses revealed dramatic increases in TNF- $\alpha$  in patients with acute vasculitis in association with Wegeners granulomatosis, in patients with systemic mastocytosis, and in the syndrome of hyperimmunoglobulin E and recurrent infections (Job's syndrome). Increases in TNF- $\alpha$  correlated well with disease activity suggesting that targeting drug development for modulation of this cytokine will have specific merit in these disease states. Normal blister exudate neutrophils synthesize large amounts of IL-8, compared with control peripheral blood neutrophils. Studies of normals and patients with excessive inflammation revealed a tight linear relationship between the level of IL-8 and the number of inflammatory neutrophils in the exudate. Exudate neutrophils synthesize IL-8 which is stored in a compartment different from specific or azurophil granules, but is co-eluted with an alkaline phosphatase rich plasma membrane fraction. Exposure of blood neutrophils to the calcium ionophore A23187 mimicked exudation and increased neutrophil IL-8 concentration 200-fold.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00614-05

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Phagocyte Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas L. Leto, Ph.D.	Investigator	LHD/NIAID
Others:	Isabelle Ossona de Mendez, Ph.D.	Visiting Scientist	LHD/NIAID
	Malathi Sathyamoorthy	IRTA Fellow	LHD/NIAID
	Cheung H. Kwong, M.D., Ph.D.	Special Volunteer	LHD/NIAID
	Anthony G. Adams, B.A.	Biologist	LHD/NIAID

COOPERATING UNITS (if any)

Ariad Pharmaceuticals, Cambridge, MA (J.S. Brugge and R. Rickles); Dept. of Chemistry, Purdue Univ. (P.S. Low); Dept. Infect. Dis., Ben-Gurion Univ, Beer Sheva, Israel (R. Levy); Dept. Biochem., Wake Forest Univ. (L.C. McPhail)

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Building 10, Rm 11N 106, Bethesda, MD, 20892

TOTAL STAFF YEARS:

4.2

PROFESSIONAL:

3.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Structure-function relationships involved in assembly and activation of NADPH oxidase (respiratory burst) have been explored both in vitro and in transfected cell models. This enzyme is an important host defense system of phagocytes responsible for production of superoxide anion and related microbicidal oxidants; oxidase deficiencies cause chronic granulomatous disease. Recent work has also focused on the oxidase as a model for protein-protein interactions relevant in diverse intra-cellular signal transduction cascades. We have established a model in which multiple interactions of conserved Src Homology 3 (SH3) domains in two cytosolic components (p47-phox and p67-phox) with proline-rich target sequences in other oxidase components mediate NADPH oxidase assembly. Both constitutive and regulated SH3 interactions have been elucidated, based on translocation and activation phenomena observed in whole cells. A tail-tail SH3 interaction between p67-phox and p47-phox affects stability of p67-phox in transfected cells, while two other p47-phox SH3 interactions were evident during oxidase activation when the functions of truncated forms of these proteins were compared. Structural determinants for SH3 domain recognition have also been investigated by gene transfer and mutagenesis; binding epitopes were compared with other well characterized SH3-peptide ligand complexes. We also initiated screening of biased peptide libraries to broaden our understanding of binding specificities of all SH3 domains of this system. We have engineered nine phosphorylation site mutations in p47-phox to explore the role of phosphorylation in regulation of SH3 function during oxidase activation. In related work, we have cloned a cDNA encoding a third cytosolic oxidase component with an SH3 motif (p40-phox), which appears to form a high molecular weight complex with the other cytosolic components. We have expressed p40-phox cDNA in a recombinant baculovirus and have affinity purified this protein through interaction with the C-terminus of p47-phox. We have also expressed most oxidase proteins in the yeast two-hybrid system and have demonstrated interactions in these vectors between p40-phox and the N-terminus of p67-phox and between p67-phox and p21-rac, a Ras-related regulator of the oxidase. These studies may be used as a structural basis for designing drugs that can inhibit this important aspect of inflammation or provide insights on other diverse signal transduction systems involving structurally related proteins.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00615-05 LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Molecular Analysis of Neutrophil Activation by Chemoattractants**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Philip M. Murphy, M.D.	Senior Investigator	LHD, NIAID
Ji-Liang Gao, Ph.D.	Visiting Associate	LHD, NIAID
Sunil Ahuja, M.D.	Visiting Associate	LHD, NIAID
H. Lee Tiffany, Ph.D.	Biologist	LHD, NIAID
Christophe Combadiere, Ph.D.	Visiting Scientist	LHD, NIAID

COOPERATING UNITS (if any)

Christine Kozak, Ph.D., LMM, NIAID  
 Elmer Becker, M.D., Ph.D., University of Connecticut Health Center  
 Richard Freer, Ph.D., Medical College of Virginia

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To understand phagocyte activation processes, we previously cloned cDNAs for the following G protein-coupled chemotactic receptors: the N-formylpeptide receptor, interleukin-8 receptor type B (an  $\alpha$  chemokine receptor), and the MIP-1 $\alpha$ /RANTES receptor (a  $\beta$  chemokine receptor, also known as CC CKR1). We also previously established that open reading frame ECRF3 of Herpesvirus saimiri encodes an interleukin-8 receptor. We have now characterized the first potent non-formylated synthetic peptide agonist for the N-formylpeptide receptor, breaking a longstanding structural constraint for the consideration of natural agonists for the receptor. Our work on chemokine receptors has led to the following recent discoveries. 1) We established that open reading frame US28 of human cytomegalovirus encodes a  $\beta$  chemokine receptor most selective for RANTES. 2) We cloned the first eosinophil-selective  $\beta$  chemokine receptor, designated CC CKR3. Its agonist rank order is MIP-1 $\alpha$ >RANTES>MIP-1 $\beta$ . 3) We showed that in addition to MIP-1 $\alpha$  and RANTES, MCP-3 is also a potent agonist for CC CKR1. CC CKR1 is the first receptor cloned for MCP-3. 4) CC CKR1 is highly expressed in monocytes, neutrophils, placenta, liver and lung. CC CKR2B is an MCP-1 receptor selectively expressed in monocytes, lung and liver. 5) We cloned a gene for a related orphan receptor CMKBRL1 that is selectively expressed in brain, neutrophils and monocytes. 6) We identified a mouse gene cluster on chromosome 9 that contains three genes related to the human genes for CC CKR1 and CC CKR3, and showed that a third related human gene probably does not exist. All three mouse genes are expressed in leukocytes, but are differentially expressed in mouse solid organs, suggesting novel targets for  $\beta$  chemokine action. Two of the genes are currently orphans, the third encodes the first functional mouse  $\beta$  chemokine receptor to be cloned. Its only known agonist is MIP-1 $\alpha$ . 7) Finally, we established the gene organization and promoter function for interleukin-8 receptors A and B. Our work indicates profound complexity in the molecular apparatus that mediates phagocyte chemotaxis. Our discovery of differential expression of chemokine receptors in phagocytes suggests they may be responsible for selective phagocyte accumulation in pathological states, making them good targets for development of cell type-selective anti-inflammatory drugs. Our discovery of two viral chemokine receptors points to new ways for studying how herpesviruses infect their hosts and evolve with them.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00644-04 LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of peripheral blood progenitors: a target for gene transfer.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Harry L. Malech, MD Deputy Laboratory Chief LHD/NIAID

Others:

Michael Mardiney, MD Clinical Associate LHD/NIAID

Steven Rafferty, PhD Visiting Fellow LHD/NIAID

Joseph Domechowski, MD Special Volunteer LHD/NIAID

Fei Li, PhD IRTA Fellow LHD/NIAID

Seema Ahuja, MD IRTA Fellow LHD/NIAID

COOPERATING UNITS (if any)

Sudhir Sekhsaria, MD IS/CP/CC; Thomas Fleisher, MD IS/CP/CC;  
Susan Leitman, MD BSS/DTM/CC

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

3.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project studies peripheral blood hematopoietic progenitors (PBHP) as a target for gene therapy of inherited diseases affecting the function of human phagocytic cells, including neutrophils, eosinophils, and monocytes. A related goal of this project is development of novel cellular therapies based on genetically engineering human PBHP and their phagocytic cell progeny to endow them with new properties to augment host defense against chronic infections with intracellular pathogens including tuberculosis and other mycobacteria infections. We have defined conditions for optimum harvest, purification and culture of the primitive human hematopoietic cells with a CD34 surface antigen phenotype. In a clinical study we determined that 10 ug/kg daily subcutaneous granulocyte colony stimulating factor (G-CSF) administered for 5 or 6 daily doses optimizes recruitment of circulating primitive CD34 progenitor cells, and that recruitment is transient, peaking on day 5. Using a antibody-magnetic bead technology developed by Baxter Healthcare we have achieved purification of 50 to 200 million CD34+ cells from donors, and used these cells for development of gene transfer therapy. In related studies we have studied optimum conditions for growth of CD34+ cells in culture using stem cell factor, IL3, IL6, plus the additional early acting factor, FLT3 resulting in expansion of very early progenitors capable of generating GEMM colonies. We have developed specific culture conditions leading to predominantly neutrophils, eosinophils or monocyte/macrophages. This will be important for studies aimed at engineering new characteristics into specific end stage phagocytes by gene transfer into progenitors. Using conditions for monocyte differentiation we introduced a gene for interferon gamma (IFN- $\gamma$ ) into these cells by targeting the CD4+ progenitors, finding that this resulted in augmentation of oxidase activity and an increase in IgG Fc receptors. In other studies, the gene for nitric oxide synthase (NOS) was successfully transferred into hematopoietic progenitor cell lines. This enzyme produces nitric oxide, an oxidant implicated in host defense against intracellular pathogens, including tuberculosis. These studies of IFN- $\gamma$  or NOS gene transfer into hematopoietic progenitors has potential as a means of augmenting monocyte host defense activities for treatment of chronic intracellular infections.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00645-04 LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene therapy for immune deficiencies.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Harry L. Malech, MD Deputy Laboratory Chief LHD/NIAID

Others:

John I. Gallin, MD Laboratory Chief LHD/NIAID

Michael Mardiney, MD Clinical Associate LHD/NIAID

Steven Rafferty, PhD Visiting Fellow LHD/NIAID

Joseph Domechowski, MD Special Volunteer LHD/NIAID

Fei Li, PhD IRTA Fellow LHD/NIAID

Wayne Weil Student IRTA LHD/NIAID

COOPERATING UNITS (if any)

Sudhir Sekhsaria, MD IS/CP/CC; Thomas Fleisher, MD IS/CP/CC; Michael Gottesman, LCB/DCBDC/NCI; Susan Leitman, MD BSS/DTM/CC; Laurence Cohen, PhD, Somatix Therapy Corp; Phillip Maples, Immunotherapy Div., Baxter Healthcare.

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.5

PROFESSIONAL:

3.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project studies gene therapy for chronic granulomatous diseases (CGD) and other inherited immune diseases affecting human phagocytes, and also studies use of gene therapy to augment phagocyte host defense against chronic intracellular infections such as tuberculosis and other mycobacterial infections. CGD are a group of 4 distinct genetic disorders with common phenotype characterized by life-threatening recurrent infections caused by failure of blood neutrophils and monocytes to produce superoxide and hydrogen peroxide. CGD results from the failure to produce any of the components of the NADPH oxidase. We now have achieved retrovirus mediated gene transfer to functionally correct all four genetic forms of CGD by transfer of normal oxidase genes into CD34+ hematopoietic myeloid progenitor cells from CGD patients resulting in full correction of superoxide production by 5% to 80% of phagocytes differentiated from the progenitors. A closed plastic bag system for large scale growth and efficient retrovirus transduction of CD34+ progenitors has been developed in collaboration with Baxter Healthcare. Large scale production of the clinical grade replication defective retrovirus substantially free of animal proteins was developed in collaboration with Somatix Therapy Corp. Also, a sensitive FACS analysis system was developed to follow functional correction of oxidase after gene transfer correction of CGD progenitors. These scientific accomplishments allowed approval by regulatory agencies of a Phase I clinical trial of gene therapy for the p47phox deficient form of autosomal recessive CGD. This clinical trial has begun and analysis of these studies is in progress. The development of an animal protein free growth and transduction regimen together with a completely closed system for growth and transduction of CD34+ blood progenitors is a major advance in enhancing safety of gene therapy and will provide a general platform for other applications of gene transfer technology based on targeting of hematopoietic progenitor cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00646-04 LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transgenic Animal Models of Human Immune Defects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Steven M. Holland, MD	Senior Staff Fellow	LHD/NIAID
Others: Sharon H. Jackson, MD	Clinical Associate	LHD/NIAID
Choh Yeung, BA	Biologist	LHD/NIAID
David M. Frucht, MD	Research Associate	LHD/NIAID
John I. Gallin, MD	Laboratory Chief	LHD/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2

PROFESSIONAL:

1.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is directed at understanding the role of specific enzyme systems in generating and maintaining host immunity. The approach taken involves the creation of mice with discrete defects in host defenses by using homologous recombination of DNA into the host genome to disrupt relevant genes. Lesions have been introduced that correspond to previously identified mutations in humans that incapacitate important host defense systems. This project relies upon a thorough understanding of the molecular and functional organization of the NADPH oxidase system and the mutations which disable it. Further, the dissection of specific phenotypes associated with the defined genotypes may provide insight into the importance of certain functional domains of the genes of the NADPH oxidase and indicate which sites are most informative for further study.

We have used the NADPH oxidase system as a paradigm for the creation of mice with a genetic defect similar to one found in humans, chronic granulomatous disease (CGD). Using homologous recombination, we have created mice by which are deficient in the p47phox gene product, a necessary component of the NADPH oxidase complex.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00647-04 LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genes and gene products as immuno-adjuvants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Steven M. Holland, MD	Senior Staff Fellow	LHD/NIAID
Others: Sharon H. Jackson, MD	Clinical Associate	LHD/NIAID
Choh Yeung, BA	Biologist	LHD/NIAID
David M. Frucht, MD	Research Associate	LHD/NIAID
John I. Gallin, MD	Laboratory Chief	LHD/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2

PROFESSIONAL:

1.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is directed at understanding, characterizing, and evaluating the role of cytokines in the pathophysiology and treatment of infectious diseases. We have used mycobacterial diseases as the system in which to examine the role of cytokines and cellular interactions, especially those of the monocyte/macrophage, in the generation of an effective granulomatous response. We hope to determine the nature of the effective granulomatous response and thereby begin to dissect out conditions in which the granulomatous response is ineffective (e.g., tuberculosis) or inappropriate (e.g., sarcoid, Crohn's disease).

We have characterized a family with ineffective granulomatous inflammation as demonstrated by disseminated *Mycobacterium avium* infection in the absence of HIV infection. These patients have low interferon gamma production due to interleukin-12 deficiency. The determination of interleukin-12 deficiency in an infectious disease confirms the role of interleukin-12 in the control of intracellular infections in humans. We have described other patients with low interferon gamma production not due to interleukin-12 deficiency, indicating that the interferon gamma generating pathway is complex. We have successfully treated these patients with subcutaneous interferon gamma. Interferon gamma is now being used in the treatment of multiple-drug resistant tuberculosis.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00648-05 LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NADPH Oxidase Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thomas L. Leto, PhD Medical Officer LHD/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project Terminated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00649-04-LHD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Phagocyte Granule Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Helene F. Rosenberg, M.D., Ph.D.	Investigator	LHD/NIAID
Kimberly D. Dyer, M.S.	Biologist, GS-11	LHD/NIAID
Jeffrey S. Handen, Ph.D.	Post-doctoral Fellow	LHD/NIAID

COOPERATING UNITS (if any)

Graduate Genetics Department, George Washington University (H. Lee Tiffany)

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.3

PROFESSIONAL:

1.3

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

A. Structure/function of ECP and EDN. Eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) are granule proteins with structural and functional homology to members of the mammalian ribonuclease gene family. We have traced the rapid molecular evolution of this gene family, and determined that the genes encoding EDN and ECP have accumulated non-silent mutations at rates exceeding those of all other functional coding sequences studied in primates, while retaining both the structural and catalytic components required for ribonuclease activity (Rosenberg et al (1995) **Nature Genetics** 10: 219-223). Interestingly, the antibacterial activity of ECP was shown to be unrelated to ribonuclease activity (Rosenberg (1995) **J. Biol. Chem.** 270: 7876-7881). We have studied the function of a representative "ancestral" sequence of the ECP/EDN gene pair; our results suggested that evolutionary constraints have promoted two novel functions--both cytotoxicity and enhanced ribonuclease activity--in the two human members of this gene family. (Rosenberg and Dyer (1995) In review)

B. Eosinophilopoiesis. We have identified conditions under which CD34+ peripheral blood progenitor cells (PBPCs) isolated from normal individuals can be induced to differentiate toward eosinophils; while faithfully replicating transcriptional events of normal eosinophilopoiesis, only three of the five granule proteins could be readily detected, suggesting the possibility of as yet unidentified eosinophilopoietic factors (Rosenberg et al (1995) In review). Interestingly, EDN synthesized by these cells was found to be hyperglycosylated, as was EDN and ECP detected in an eosinophilic variant of the promyelocytic leukemia cell line, HL-60 (Tiffany et al (1995) **J. Leuk. Biol.** In press). Additionally, we have identified a functional promoter of the EDN gene, and shown that gene expression requires cooperation between the promoter and intronic enhancer elements, several of which may function only in hematopoietic cells (Tiffany and Rosenberg (1995) In review).

C. Charcot-Leyden crystal protein (CLC). Molecular cloning suggested a relationship between the eosinophil protein, CLC, and S-type animal lectins. Guided by this observation, we have shown that CLC can function as a lactose binding protein (Dyer and Rosenberg (1995) In preparation).



# Laboratory of Immunogenetics

## 1995 Annual Report

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## **LABORATORY OF IMMUNOGENETICS**

National Institute of Allergy and Infectious Diseases  
October 1, 1994 to September 30, 1994

### **RESEARCH IN PROGRESS**

Research in the Laboratory of Immunogenetics currently includes studies of natural killer (NK) cell receptors, the genetics of human T cell receptors (TCR), antigen presentation to CD4 T cells, and animal models of retrovirus infection. Most of this work has exploited molecular approaches to answer questions of immunological interest.

The isolation of molecular clones for a family of inhibitory receptors expressed by NK cells has opened the way to a molecular dissection of these receptors and their function. The complete molecular mapping of the gene complex encoding variable regions of the TCR beta chains (TCRBV) has made it possible to assess TCR gene usage in healthy and pathological immune responses. A new processing pathway for the presentation of antigens to CD4 T cells has been discovered. The successful isolation of infectious molecular clones of HTLV-1, and the susceptibility of rabbits to HTLV-1 infection, form the basis of a useful animal model for adult T cell leukemia. Finally, new antibodies specific for rabbit molecules of immunological importance, and new immune response genes have been characterized in order to make the rabbit a better animal model to study immune disorders, including infection by HIV-1. This overview details the research highlights of LIG.

#### **The Natural Killer Cell Inhibitory Receptor**

**Molecular Clones of the p58 Natural Killer Cell Receptor Reveal Ig-related Molecules with Diversity in both the Extra- and Intracellular Domains.** Recognition of major histocompatibility class I molecules on target cells by NK cells confers selective protection from NK-mediated lysis. Crosslinking of the p58 NK receptor, involved in the recognition of HLA-C alleles, delivers a negative signal that prevents target cell lysis. Molecular cloning of the p58 NK receptor revealed a new member of the immunoglobulin superfamily. Five distinct p58 receptors, with sequence diversity in the Ig-related domains, were identified in a single individual. All NK clones tested expressed at least one p58 member. Three different types of transmembrane and cytoplasmic domains exist, even among receptors with closely related extracellular domains. These data revealed a repertoire of NK cells with clonally distributed p58 receptors exhibiting diversity in both extracellular and intracellular domains (Wagtmann, Burshtyn, Rajagopalan).

**Peptide Specificity in the Recognition of MHC Class I by Natural Killer Cell Clones.** Recognition by NK cells of major histocompatibility complex (MHC) class I molecules on target cells inhibits NK-mediated lysis. This study revealed that inhibition of NK clones by HLA-B\*2705 molecules mutated at single amino acids in the peptide-binding site varied among HLA-B\*2705-specific NK clones. Furthermore, a subset of such NK clones was inhibited by only one of several self peptides loaded onto HLA-



B\*2705 molecules expressed in peptide transporter-deficient cells, showing that recognition was peptide-specific. These data demonstrate that specific self peptides, complexed with MHC class I, provide protection from NK-mediated lysis. These results can also explain the heterogeneity among NK clones in their ability to lyse virus-infected targets: the loss of protection from NK lysis caused by virus infection no longer implies replacement of most endogenous peptides but could occur through interference with the formation of specific class I-peptide complexes (**Peruzzi**).

### **The Functional Human TCR Repertoire**

Genes encoded within the MHC and TCR gene complexes play important roles in immune responses and in susceptibility to autoimmune diseases. The contribution of individual gene products within these gene complexes and the mechanisms by which they function remain obscure. In the present studies, the extent of the germline repertoire of TCR beta (TCRB) genes was defined and diversity of T cells in certain immune responses was characterized. Examination of the germline TCRB variable (TCRBV) gene repertoire revealed 64 genes of which 51 are functional. Polymorphic gene segments and regions within the gene complex showing genetic variation were identified; these include two frequently occurring insertion/deletion related polymorphisms (IDRP). The IDRP located in the TCRBV region contained three genes; BV7S3, BV9S2(P), and a second, identical, copy of BV13S2. The inserted region is >98% identical to a segment present in all individuals which suggests that genetic mechanisms for its origin may include unequal crossing over, gene conversion like events, insertion and deletion of short segments of DNA and mutation (**Zhao, Robinson**). Six orphan BV genes were mapped to chromosome 9 outside of the TCRB gene complex. The orphan genes and 7 BV genes were found to be pseudogenes (**Currier**) and three BV genes were found to have null or nonfunctional alleles (**Deulofeut, Barron, Currier**). The utilized TCR repertoire was examined by analysis of CDR3 length diversity (spectratyping), sequencing, and semiquantitative PCR as well as by the use of monoclonal antibodies to TCRV chains. T cells responding to Hepatitis B surface antigen showed oligoclonal populations of T cells for several BV families. The predominant BV families and CDR3 lengths used were similar among different responder individuals (**Deulofeut**). Activated cells from patients with autoimmune diseases were analyzed in a similar fashion. Patients with Kawasaki's disease showed restricted expansion of T cells. Several TCRBV families had one predominant CDR3 length; however, sequence analysis indicated significant sequence diversity (**Barron**). Detailed knowledge of the extent and diversity of the germline TCR repertoire will greatly facilitate our ability to understand the role of TCR genes in immune responses in normal and disease states.

### **A New Pathway for Antigen Presentation to CD4 T cells**

Antigen presentation mediated by recycling of surface HLA-DR molecules. Class II histocompatibility molecules associate with peptides derived from antigens that are processed in endocytic compartments. Antigen presentation to class II-restricted T cells generally requires newly synthesized class II molecules, associated invariant chain, and HLA-DM. Exceptions to these rules have been reported but without description of an underlying mechanism. We have demonstrated that presentation of



immunodominant epitopes in the haemagglutinin protein of influenza virus and in myelin basic protein correlated strictly with recycling of surface HLA-DR molecules. Truncation of either one of the  $\alpha$  or  $\beta$  cytoplasmic tails virtually eliminated internalization of HLA-DR molecules and presentation of haemagglutinin from inactive virus particles. In contrast, the invariant chain-dependent presentation of matrix antigen from the same virus particles was unaffected by these truncations. Thus, HLA-DR cytoplasmic tails are not required for the conventional presentation pathway but jointly contribute a signal for an alternative pathway involving internalization of HLA-DR molecules. This pathway may be useful for the presentation of antigens that are rapidly degraded upon uptake into antigen-presenting cells (**Long, Rojo**).

### **Infectious Molecular Clones of HTLV-I from Rabbit Cell Lines**

Susceptibility of rabbits to infection with HTLV-I is well documented; infection is readily transferred and is marked by seroconversion and by the presence of infected lymphocytes in circulation. Except in rare circumstances, rabbit HTLV-I infection, as in the majority of human infections, causes no overt disease. An exception to this was a rabbit cell line (RH/K34) derived by in vitro infection; inoculation with this line reproducibly caused fatal leukemic-like disease in rabbits. Genetic matching between the recipient to the inoculated cell line did not influence the outcome of infection. A detailed description of HTLV-I induced disease in rabbits revealed many similarities to human disease and the study was supplemented by extensive controls using a variety of HTLV-I inocula and control cell lines (**Simpson**). Several rabbits survived the lethal injection and all of these were shown to have significant levels of antibody directed against hsp 70. Active immunization with hsp 70 (but not passive administration of Ab from immunized rabbits) prevented death in rabbits given a lethal dose of RH/K34 cells (**Mahana**). Characterization of the lethal cell line and determination of the complete sequence of the integrated provirus revealed no obvious differences from HTLV-I lines that cause asymptomatic infection. It was observed that exposure of peripheral blood mononuclear cells (PBMC) to cell free virus from the lethal line causes profound apoptosis of T lymphocytes while virus from a non lethal line mediates dose dependent lymphoid cell proliferation (**Leno**). Furthermore, thymuses of rabbits given lethal inoculum were atrophied, just as were thymuses taken at autopsy from ATLL patients. Recently, chimeric and mutagenized infectious HTLV-I clones have been constructed (**Zhao**). It is now possible to test these clones in the in vivo and in vitro models and to monitor host responses to them. This approach has the possibility to yield new information concerning roles for specific virus genes in the variable pathogenicity of HTLV-I in human infection.

These recent data along with reports concerning the nature of HTLV-I disease in humans suggest a mechanism for development of aggressive leukemic disease (ATLL) in individuals following many years of asymptomatic infection. The in vivo studies with the lethal HTLV-I inoculum and the reports from autopsy of ATLL patients indicate that thymic atrophy is a common feature. In addition, immunodeficiency typically accompanies late stage HTLV-I disease. The observation that apoptosis occurs in the T cell population when PBMC are exposed to virus from the lethal cell



line opens the possibility that development of leukemia is made possible by impaired immune surveillance of the HTLV-I infected cells by host T cells. The obvious question of why the leukemic cells, which are themselves T cells, do not succumb to apoptosis may be answered by recent data indicating that the HTLV-I tax protein prevents apoptosis induced by HIV-1. Whether this protection extends to HTLV-I induced death can and will be tested. An additional finding was that rabbits making antibodies to hsp-70 did not develop lethal leukemia and that injection with all cell lines, except the lethal RH/K34 (even when lethally irradiated or at sub lethal doses of live cells), induced the anti hsp response. The fact that passive administration of rabbit anti hsp did not provide protection to the lethal inoculum suggests that cellular immunity to hsp is needed. While the development of lethal disease in HTLV-I infected individuals is a lengthy and complex process, the sum of our data along with published information, suggests that the switch from asymptomatic infection to aggressive leukemia may result from acquisition in the infected cell of the ability to produce apoptosis in host T cells. This property would allow the infected cell to elude immune mechanisms that keep the infection under control. Advances made with the rabbit HTLV-I disease model should facilitate a search for viral and host cell factors contributing to this ability to induce T cell apoptosis.

#### **CD4 and its role in Rabbit HIV Infection**

Studies of cell surface markers for rabbit lymphoid cell populations have emphasized rabbit T cells with special reference to those involved in retrovirus infection. The rabbit CD4 (RbCD4) gene was used in transfection experiments to compare its efficacy as a receptor for HIV-I to human CD4 which is known to bind the env protein of HIV with high affinity. RbCD4 transfectants of human and mouse cell lines were compared with these lines expressing human CD4. RbCD4 proved to be a poor receptor for HIV-1 and therefore rabbits transgenic for human CD4 were developed. Transgenic animals have been obtained and shown to express HuCD4 on appropriate T lymphocyte populations and in a development specific fashion. Infections of PBMC from the transgenics with HIV-1 are more productive than normal rabbit infection. PBMC from the transgenic rabbits undergo rapid apoptosis in the presence of cell free HIV-I. Normal rabbit PBMC also undergo apoptosis when exposed to cell free HIV-1 but the effect is considerably slower and of a lesser magnitude than the transgenic cells (**Leno**). The transgenic rabbits are being infected in vivo with different HIV isolates and the presence of virus and the effects on Hu CD4 bearing cells is being monitored.

The realization that barriers (in addition to receptor function) to efficient HIV infection of rabbit cells exist has prompted study of other stages in the viral replication process in rabbit versus human cell lines. Studies in progress involve the use of reporter gene constructs to test LTR function and to assess the function of the regulatory genes tat and rev in rabbit cells. Cell lines transfected with HIV-1 molecular clones have been used to circumvent the early steps in viral replication and to demonstrate that infectious viral particles are assembled and transported in rabbit cells. In the course of these studies, one rabbit cell line was found to contain an endogenous retrovirus the expression of which was induced by treatment with various agents, including HIV-1 tat (**Hague**). Characterization of this virus is being pursued





because presence of an endogenous rabbit retrovirus may affect the results of our in vitro and in vivo studies. This is especially relevant in light of findings that an AIDS-like disease resulted in recipients of blood from HIV infected donor rabbits without clear signs of HIV-1 infection.

### **The Rabbit MHC**

The major histocompatibility complex of the rabbit (RLA) differs from the well characterized MHC regions of human and mouse in that class II DR genes are adjacent to class I genes with no interposition of class III genes. Current investigations involve derivation of a series of probes to map the complex by pulse field electrophoresis in order to ascertain the complete map of class I and to define the extremes of class III which was earlier shown to be genetically linked to the MHC. In other studies the nonpolymorphic gene encoding the class II DO beta chain has been expressed in an E. coli protein system and the product used to prepare monoclonal antibodies (**Samaan, Mahana**).



# LABORATORY OF IMMUNOGENETICS

## ANNUAL REPORT

October 1, 1994 to September 30, 1995

### HONORS AND AWARDS

Dr. Thomas Kindt, Chief of the Laboratory of Immunogenetics was an invited participant and speaker at the Second African Immunology Conference held in Nairobi, Kenya. Laboratory data were presented at the meeting of the Laboratory of Tumor Cell Biology, at the ASM AIDS meeting, at a Keystone conference on apoptosis, at the Children's Hospital at the University of Cincinnati and at a symposium organized to honor Dr. Richard M. Krause at the Fogarty International center. He was also invited to present an NIH special lecture. Dr. Kindt serves on the publication committee of the American Association of Immunologists and was named to the FASEB publication committee. He is an Associate Editor of the *FASEB Journal* and North American regional editor of *Research in Immunology*. and was invited to serve as editor of the *Scandinavian Journal of Immunology*. Dr. Kindt serves on the Genetics Advisory Committee of the NIH Veterinary Resource Branch and has assisted in Planning an International Conference on Immunology and Aging. Dr. Kindt continues to serve on the scientific advisory boards of Oncor Inc., Gaithersburg MD, and Innovir Laboratories Inc., New York, NY.

Dr. Eric Long, head of the Molecular and Cellular Immunology Section of LIG, was an invited speaker at the Immunology Seminar Series at Harvard Medical School, and presented seminars at the Blood Center of Southeastern Wisconsin and at the University of Barcelona, Spain. He presented a paper at a workshop during a Keystone Symposium. He was invited to chair a Minisymposium on "Antigen Processing and Presentation on MHC Class II Molecules" during the FASEB 95 meeting in Atlanta. He was invited to give a talk at a Symposium on "MHC Class II Synthesis and Assembly", and deliver a paper at a workshop on "NK cells" at the International Congress of Immunology in San Francisco. He was also invited to participate in an international workshop on "NK cell receptors and recognition of the MHC antigens" in Madrid, Spain. Dr. Long serves on the Advisory Committee for the Howard Hughes Medical Institute - National Institutes of Health Research Scholars Program.

In the past year, Dr. Robinson organized the research program for the 20th annual meeting of the American Society for Histocompatibility and Immunogenetics. She chaired a plenary session on programmed cell death, and served on the Scientific Affairs committee. She serves on the editorial board of Human Immunology and participated in a special committee to establish a new section to the journal. She served on Ph.D. committees for two candidates, one at University of Texas Southwestern Medical Center in Dallas and another at Georgetown University in Washington DC. She was an invited speaker at Cornell University and at University



of Texas Southwestern Medical Center. Dr. Robinson was elected Woman Scientists Advisor and serves on the Coordinating Committee on Research on Women's Health.

Dr. Karyl Barron was an invited speaker at the Third Brazilian Congress of Pediatric Rheumatology in Sao Paulo, Brazil and at the Brazilian Society of Pediatrics in Rio de Janeiro. She presented Grand Rounds at Universidade do Estado do Rio de Janeiro and another Grand Rounds for the Perinatology Department at George Washington University.



# **LABORATORY OF IMMUNOGENETICS**

## **ANNUAL REPORT**

**October 1, 1994 to September 30, 1995**

### **ADMINISTRATIVE REPORT**

The Laboratory of Immunogenetics occupies laboratory and animal space in the NIAID Twinbrook II facility in Rockville, Maryland. The laboratory continues to maintain a contract facility at Spring Valley Laboratories for animals infected with human retroviruses.

Dr. Kindt, Chief, of LIG, was appointed Director, Division of Intramural Research for NIAID. He will retain position as Chief, LIG and Dr. Long will act as Chief in his absence.

The Unit headed by Dr. Mary Ann Robinson, previously incorporated in the Immunogenetics Research Section was given Section status, with the name "Human Immunogenetics Research Section". Dr. Mary Ann Robinson was promoted to Section Head.

In the past year, the Immunogenetics Research Section was joined by Dr. Mei Han from the Central Institute for Electron Microscopic Research in Tokyo, Japan and Dr. Lisa Carlton from the University of North Carolina who will both work with Dr. Kindt.

The Molecular and Cellular Immunology Section was joined by Dr. Deborah Burshtyn from the University of Toronto, Canada, and Dr. Sumati Rajagopalan from Harvard Medical School, who will both work with Dr. Long. Dr. Marta Peruzzi was converted from Special Volunteer to a Visiting Fellow position.

Departures include Dr. Sungae Cho from the Immunogenetics Research Section who will pursue postdoctoral work at the University of California in Los Angeles, and three postdoctoral fellows from the Molecular and Cellular Immunology Section: Dr. Valérie Pinet who assumed a position at the University of Montpellier, France, Dr. Maryse Guéguen who joined the Ludwig Cancer Research Institute in Brussels, Belgium, and Dr. Petronella Warmerdam who joined the University of Leuven, Belgium. In addition, Dr. Mauro Malnati, a Visiting Associate in the Molecular and Cellular Immunology Section has assumed an independent position at the Department of Biotechnology in the San Raffaele Institute in Milan, Italy.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01-AI-00166-18

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas J. Kindt	Chief, LIG	LIG, NIAID
Other:	A. Samaan	Visiting Fellow	LIG, NIAID
	W. Mahana	Visiting Fellow	LIG, NIAID
	M. Han	Visiting Fellow	LIG, NIAID

COOPERATING UNITS (if any)

Rafick P. Sekaly, Ph.D., Insitut de Recherches Cliniques de Montreal, Director  
 Laboraotry of Immunology

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Reseach Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL STAFF YEARS:

3.9

PROFESSIONAL:

2.4

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigations of the major histocompatibility complex of the rabbit (RLA) have led to identification of a nonpolymorphic gene encoding the class II DO beta chain. RLA-DOB is nearly identical to its human counterpart and has large differences from other class II beta chains. The product is expressed only on mature B cells and to a limited extent in thymus. In order to study the the function of the DOB product, monoclonal and polyclonal antibodies against human and rabbit DOB are in preparation. The antibodies will be used to localize DOB and to isolate the DOB complex (putatively DN/DO) and eventually to ascertain whether a peptide or other molecule is associated with the molecular complex. Efforts to construct a complete physical map of the rabbit MHC are in progress and attempts to order the various MHC regions in the rabbit continue. Previous results showed that class II and class I regions were adjacent with no interposition of Class III genes. Studies of class III gene prompted a study of heat shock protein 70 which is expressed on HTLV-I infected cells. Anti-heat shock protein (HSP) antibodies were observed in the sera of rabbits after inoculation with the HTLV-I cell lines. One cell line RH/K34, which failed to raise a response to HSP70, was shown subsequently to cause lethal leukemia when live cells were injected into unrelated outbred rabbits. Injection of sublethal doses of this line failed to elicit anti HSP although injection of cell free virus from the line did so. Rabbits with detectable levels of anti HSP70 antibodies prior to challenge with lethal doses of live RH/K34 cells were resistant to its lethal effects suggesting that HSP immunity (which is autoimmunity) may influence the outcome of RH/K34 pathogenicity and may have general implications to viral immunity. In order to study the interaction between HSP and HTLV-1 infection, specific polyclonal and monoclonal antibodies against HSP 70 and HSP 90 will be tested to determine whether disease and/ or infection can be inhibited by passive or active immunization.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00168-18

PERIOD COVERED

September 1, 1994 to October 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Surface Markers of Rabbit Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.J. Kindt	Chief	LIG, NIAID
Others:	Bishop Hague	Senior Staff Fellow	LIG, NIAID
	Michel Leno	Visiting Fellow	LIG, NIAID
	Wahib Mhana	Visiting Fellow	LIG, NIAID

COOPERATING UNITS (if any)

Drs. B. Snyder, N. Rudolph, P. Leibowitz, TSI Inc., Worcester, MA  
Dr. J. Michael Wilkinson, Jeannette Blackford, The Wellcome Trust, London

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:

3.3

PROFESSIONAL:

1.8

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

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☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of cell surface markers for rabbit lymphoid cell populations have emphasized rabbit T cells with special reference to those involved in retrovirus infection. We have previously derived HTLV-1 transformed cell lines that differ in their pathogenic potential: one cell line (RH/K34) causes an ATLL like disease and death in rabbits, whereas the other (RH/K30) induces a latent infection. The lethal cell line (RH/K34) induces thymocyte depletion and virus produced by this cell line causes apoptosis of human and rabbit lymphocytes. In contrast, the virus purified from the RH/K30 cell line induces proliferation. Rabbit CD4 (RbCD4) transfectants of human and mouse cell lines were compared with these lines expressing human CD4 (HuCD4). RbCD4 proved to be a poor receptor for HIV-1 and therefore rabbits transgenic for human CD4 were developed. Transgenic animals have been obtained and shown to express HuCD4 on appropriate T lymphocyte populations and in a developmentally specific fashion. Infections of PBMC from the transgenics with HIV-1 are more productive than normal rabbit infection. Experiments using human, rabbit or mouse cell lines transfected with HUCD4 or RbCD4, and infected with inactivated or non-inactivated HIV-1 indicate that productive HIV-1 infection is necessary to trigger the apoptotic mechanism. The expression of FAS antigen was significantly increased in HIV-1 infected PBMC from HuCD4 transgenic, but not normal rabbits. Other rabbit lymphoid cell markers investigated include the gd T cell receptors, CD8 $\beta$  and IL-2R $\alpha$ . Transcripts of the delta TCR gene occurred in two distinct forms, a duplication of exon 2 of the constant region was found in the larger message. This was shown to result from a genetic polymorphism in which the gene encoding TCR Cdelta was duplicated along with some flanking intron sequences. This insertion deletion polymorphism (IDRP) was shown to be inherited as an autosomal codominant trait and animals homozygous for the inserted form had levels of gd T cells in their peripheral blood that were equal to rabbits homozygous for the shorter form. Present studies continue to characterize T cell surface markers that may play a role in pathogenic effects of HTLV-I and HIV-1 infected cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00170-18

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Functional Analysis of Human Class II Histocompatibility Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Eric O. Long

Section Head

LIG, NIAID

Others: S. Rojo

Visiting Fellow

LIG, NIAID

COOPERATING UNITS (if any)

M. Vergelli, R. Martin, Neuroimmunology Branch, NINDS  
O. Bakke, University of Oslo, Norway

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular and Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:

3.1

PROFESSIONAL:

1.6

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Most immune responses occur only if a CD4 T lymphocyte recognizes foreign antigen in association with a self class II molecule of the major histocompatibility complex (MHC). According to the books, antigen presentation to CD4<sup>+</sup> T cells involves antigen uptake into endocytic compartments where unfolded proteins meet with newly synthesized MHC class II molecules. Furthermore, these class II molecules are transported there directly by their association with the invariant chain. In contrast to this simplified view, the Section has discovered a multiplicity of pathways for class II-restricted antigen presentation. First, a new transport pathway for class II molecules to antigen processing compartments was described: a cohort of newly synthesized MHC class II molecules is transported directly to the cell surface, from where it is retrieved by a very efficient internalization signal in the cytoplasmic tail of the invariant chain. Interestingly, there is selectivity in this transport pathway: only class II molecules associated with the p33 form of the invariant chain are detected at the cell surface. Class II molecules associated with the p35 form of the invariant chain are transported directly to antigen processing compartments from the trans-Golgi. Second, a new pathway for the presentation of cytosolic proteins by class II molecules was identified. This endogenous pathway has been distinguished by genetic and biochemical approaches from the processing pathway for class I-restricted antigen presentation. In contrast to the class I pathway, this endogenous pathway for class II-restricted presentation involves only long-lived cytosolic antigen. Finally, several immunologically relevant antigens are presented by the recycling of mature cell surface MHC class II molecules. This pathway may be useful for the presentation of antigens that are rapidly degraded upon uptake into antigen-presenting cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01-AI-00171-18

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Infection of Rabbits with Human Immunodeficiency Virus 1

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. J. Kindt	Chief	LIG, NIAID
Other:	B.F. Hague	Staff Fellow	LIG, NIAID
	R.M. Simpson	Expert	LIG, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

1.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Infection in rabbits injected with HIV-1 has been detected by seroconversion, detection of virus in cells and organs using PCR and *in situ* hybridization, and by isolation of virus from PBMC. While the fact that rabbits can be infected with HIV is promising, the slow course of infection, difficulty in isolation of virus and the absence of consistent disease signs in infected rabbits have limited utility of this model for AIDS. Information gained in recent efforts aim to improve the model suggest that infection in the rabbit may be enhanced in several ways. Adaptation of HIV to grow more efficiently in rabbits has been attempted by long term *in vivo* passage; injection of as little as 1 ml of blood from animals receiving splenocytes from a rabbit infected with HIV-1 and held for 2.3 years has resulted in lymphocytopenia, CD4 T cell depletion and for 2 of 12 rabbits, death. Initial results from structural analyses of HIV-1 amplified from a spleen cell recipient shows nucleotide sequence identity of 85% to the original input isolate, HIV<sub>Lai</sub>. In order to determine whether the slow progression of infection in rabbits is rooted in problems with transcription of viral DNA, CAT reporter gene constructs utilizing the 5' or 3' LTRs from HIV-1 to provide the promoter or 3' processing signals were developed. These are being used to compare LTR-mediated gene expression in rabbit and human cells. There are no apparent defects in promoter or processing of genes flanked by HIV-1 LTR's in rabbit cells. In subsequent experiments, molecular clones of HIV-1 were used in transfection experiments to assess production of infectious virus in rabbit as compared to human cell lines. Both species produced virus that appeared normal by all criteria, including ability to infect human cells. The rabbit lines produced a somewhat higher ratio of defective particles. One rabbit cell line has been found to produce a retroviral particle upon induction by iododeoxyuridine or transfection of the HIV-1 tat gene. This virus may represent a novel rabbit retrovirus that may play a role in the infection of rabbits with HIV-1.





<div style="text-align: right; padding-right: 10px;"> PROJECT NUMBER   Z01-AI-00180-17 </div>	
PERIOD COVERED October 1, 1994 to September 30, 1995	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Properties of Transformed Rabbit Cell Lines	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)	
PI: T.J. Kindt Other: T.M. Zhao R.M. Simpson L.D. Carlton M.A. Robinson	Chief Staff Fellow Expert IRTA Fellow Section head LIG, NIAID LIG, NIAID LIG, NIAID LIG, NIAID LIG, NIAID
COOPERATING UNITS (if any)	
LAB/BRANCH Laboratory of Immunogenetics	
SECTION Immunogenetics Reserach Section	
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, Rockville, MD 20852	
TOTAL STAFF YEARS: 5.0	PROFESSIONAL: 2.7
OTHER: 2.3	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A series of HTLV-I cell lines were prepared by <i>in vitro</i> transformation of rabbit blood mononuclear cells (PBMC) using HTLV-I. Animals injected with certain infected lines (e.g.RH/K34) developed adult T cell leukemia-lymphoma-like disease (ATLL). The majority of rabbit HTLV-I T cell lines (e.g.RH/K30), including several derived in the same fashion as the lethal line, cause no overt disease. Results of a detailed study may shed light on why HTLV-I infection in humans causes serious disease in only a small percentage of infected individuals. The laboratory has succeeded in defining an HTLV-I molecular clones to use for a study of specific viral genes. Several T cell and fibroblast lines transfected with the clone derived from RH/K30 expressed HTLV-I p24 gag protein at levels comparable to the donor RH/K30 line. A transfected rabbit cell line, RL-5/K30 was able to transmit HTLV-I infection as measured by both <i>in vitro</i> and <i>in vivo</i> assays. Rabbit and human lymphocytes cocultured with irradiated RL-5/K30 cells became infected with HTLV-I. Rabbits injected with RL-5/K30 cells produced an HTLV-I antibody response, and HTLV-I was isolated from PBMC beginning 3 months after injection. In order to study HTLV-I factors involved in persistence of viral protein expression, chimeric molecular clones have been constructed by shuffling genes of K30 and K34. An initial result implies that the viral genes other than the LTR region are important for maintenance of p24 expression in transfected RL-5 cells and assignment of the precise region involved in viral expression will be addressed by substituting each of the differences between K30 and K34 in turn. Ability of the molecular clones to infect rabbits <i>in vivo</i>, is being tested by direct intramuscular DNA inoculation. HTLV-I antibodies were detected in the sera obtained from the rabbits injected with K30 plasmid by two months postinjection, HTLV-I specific sequences were detected by PCR from rabbit PBMC DNA and HTLV-I virus was isolated from PBMC culture indicating infection was achieved. Availability of such clones will facilitate functional studies of HTLV-I genes and gene products.</p>	



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01-AI-00389-11

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genotype Analyses of HLA and TCR Genes in Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mary Ann Robinson	Section Head	LIG, NIAID
Other: T.M. Zhao	Staff Fellow	LIG, NIAID
H.E. Deulofeut	Visiting Fellow	LIG, NIAID
K.S. Barron	IPA	LIG, NIAID
J.R. Currier	Visiting Fellow	LIG, NIAID

COOPERATING UNITS (if any)

Jack Gorski, Ph.D., Milwaukee Blood Center, John Gerin, Ph.D, Georgetown University, A. A. Ansari, M.D., Emory University

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Human Immunogenetics Reserach Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:

5.2

PROFESSIONAL:

3.5

OTHER:

1.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Genes encoded within the major histocompatibility complex (MHC) and T cell antigen receptor (TCR) gene complexes play important roles in immune responses and in susceptibility to autoimmune diseases. The contribution of individual gene products within these gene complexes and the mechanisms by which they function remain obscure. In the present studies, the extent of the germline TCRB repertoire was defined and diversity of T cells in certain immune responses was characterized. Examination of the germline TCRBV repertoire revealed 64 genes of which 51 are functional. Polymorphic gene segments and regions within the gene complex showing genetic variation were identified; these include two frequently occurring insertion/deletion related polymorphisms (IDRP). The IDRP located in the TCRBV region contained three genes; BV7S3, BV9S2(P), and a second, identical, copy of BV13S2. The inserted region is >98% identical to a segment present in all individuals which suggests that genetic mechanisms for its origin may include unequal crossing over, gene conversion like events, insertion and deletion of short segments of DNA and mutation. Six orphon BV genes were mapped to chromosome 9 outside of the gene complex. The orphon genes and seven genes encoded within the TCRB gene complex are pseudogenes and three BV genes were found to have null or nonfunctional alleles. The utilized TCR repertoire was examined by analysis of CDR3 length diversity (spectratyping), sequencing, and semiquantitative PCR as well as by the use of monoclonal antibodies to TCRV chains. T cells responding to Hepatitis B surface antigen showed oligoclonal populations of T cells for several BV families. The predominant BV families and CDR3 lengths used were similar among different responder individuals. Activated cells from patients with autoimmune diseases were analyzed in a similar fashion. Patients with Kawasaki's disease showed restricted expansion of T cells. Several TCRBV families had one predominant CDR3 length; however, sequence analysis indicated significant sequence diversity. Detailed knowledge of the extent and diversity of the germline TCR repertoire will greatly facilitate our ability to understand the role of TCR genes in immune responses in normal and disease states.



<div style="text-align: right; padding-right: 10px;"> PROJECT NUMBER   Z01-AI-00525-08 </div>																		
PERIOD COVERED October 1, 1994 to September 30, 1995																		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Analysis of Human Natural Killer Cells																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI</td> <td style="width: 40%;">Eric O. Long</td> <td style="width: 30%;">Section Head</td> <td style="width: 15%;">LIG, NIAID</td> </tr> <tr> <td rowspan="4">Others:</td> <td>Nicolai Wagtmann</td> <td>Visiting Fellow</td> <td>LIG, NIAID</td> </tr> <tr> <td>Marta Peruzzi</td> <td>Visiting Fellow</td> <td>LIG, NIAID</td> </tr> <tr> <td>Deborah Burshtyn</td> <td>Visiting Fellow</td> <td>LIG, NIAID</td> </tr> <tr> <td>Sumati Rajagopalan</td> <td>Supplemental IRTA</td> <td>LIG, NIAID</td> </tr> </table>		PI	Eric O. Long	Section Head	LIG, NIAID	Others:	Nicolai Wagtmann	Visiting Fellow	LIG, NIAID	Marta Peruzzi	Visiting Fellow	LIG, NIAID	Deborah Burshtyn	Visiting Fellow	LIG, NIAID	Sumati Rajagopalan	Supplemental IRTA	LIG, NIAID
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TOTAL STAFF YEARS: 6.4	PROFESSIONAL: 4.4	OTHER: 2																
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Natural Killer (NK) cells play an important role in the control of viral infection before the establishment of a specific cytolytic T cell response mediated by CD3-positive MHC-restricted T cells. NK cells are also involved in the rejection of bone marrow transplants. Natural Killer cells kill target cells unless they receive a negative signal from a receptor that recognizes MHC class I molecule on target cells. The Section discovered that recognition of HLA-B27 molecules by human NK clones was not only peptide-dependent, but also peptide-specific. This provides a new conceptual framework in which to study the complex recognition of virus-infected cells by NK clones. In addition, the Section has isolated molecular clones for a family of inhibitory NK receptors involved in the recognition of HLA-C alleles. Surprisingly, these NK receptors belong to the immunoglobulin superfamily and exhibit an interesting type of dual diversity. In addition to sequence differences in the extracellular domains, these receptors exist with different types of cytoplasmic tails, even on molecules that have closely related Ig domains. A functional reconstitution system was developed and used to demonstrate that individual receptors with the longer form of cytoplasmic tail provided both the specificity for MHC class I molecules on target cells, and the negative signal that inhibits NK-mediated lysis of target cells.</p>																		



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1995 Annual Report  
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PHS-NIH  
Summary Statement  
Office of the Chief  
Laboratory of Immunology  
October 1, 1994 through September 30, 1995

## Introduction

The goals of the Laboratory of Immunology (LI) are the elucidation of the fundamental mechanisms of the immune system; the application of the resultant knowledge to the understanding of the pathogenesis of immunologic disorders such as autoimmunity, immunodeficiency, and allergic diseases; and the development of new approaches to immunization that would be useful in vaccine development. LI scientists have made important progress toward these ends through the application of powerful new technologies that have revolutionized modern biomedical science. These include techniques of contemporary molecular biology, cell biology, protein biochemistry, and structural biology, preparation and study of transgenic and gene-targeted mice, development and use of monoclonal antibodies, cloning and genetic manipulation of normal and transformed lymphocytes, and flow cytometric analysis and sorting. Investigations using these methods have provided answers to many major basic questions in immunology. This new knowledge is contributing to important advances in the control of infectious diseases and cancer, as well as to efforts to prevent and treat diseases of the immune system and disorders caused by the action of immune cells and antibodies.

## Recent Accomplishments of Laboratory of Immunology Scientists

### T Cell Differentiation in the Thymus

#### Regulation of Thymocyte Development at the Uncommitted Precursor and DN to DP Stages

T cell development in the thymus involves a large number of steps from the commitment of a multipotent, receptor-negative precursor through the committed CD4<sup>+</sup>CD8<sup>+</sup>TCR<sup>low</sup> state to the mature CD4 or CD8 (SP) stage. Previous studies showed that a c-kit<sup>+</sup>, NK1.1<sup>+</sup>, TCR<sup>-</sup> subpopulation of thymocytes could develop into NK, T, or B cells in the proper environment. New studies have now shown that the pro-inflammatory cytokines IL-1 $\beta$  and TNF $\alpha$  together contribute to restricting these multipotent precursor cells to the T lineage, coincident with the induction of expression of the CD25 (IL-2R $\alpha$ ) protein. Thus, the Pgp-1<sup>low</sup>, CD25<sup>+</sup> stage appears to be the first at which a thymocyte is restricted exclusively to the T cell developmental pathway. The next major developmental state is characterized by the CD4<sup>+</sup>CD8<sup>+</sup>TCR<sup>low</sup> phenotype. Previous studies have indicated that movement from the CD4<sup>+</sup>CD8<sup>+</sup>TCR<sup>-</sup> to CD4<sup>+</sup>CD8<sup>+</sup>TCR<sup>low</sup> (DP) stage depends on expression of a functional TCR  $\beta$  chain, and signaling via p56<sup>lck</sup>. Unexpectedly, it was found that low level irradiation of RAG <sup>-/-</sup> mice that cannot produce a functional rearranged  $\beta$  chain gene resulted in DP development. Breeding studies suggest that this effect is related to the function of the p53 proto-oncogene, in that RAG <sup>-/-</sup>, p53 <sup>-/-</sup> doubly defective mice spontaneously develop DP thymocytes, in the absence of irradiation. These studies identify key cytokine signals involved in T lineage commitment, point to IL2R $\alpha$  chain expression as defining a crucial stage in T cell development, and provide evidence that p53 may serve a gate-keeper function for a subsequent key thymocyte differentiation step (J. Zúñiga-Pflücker, D. Jiang, and M. J. Lenardo, Molecular Development of the Immune System Section, LI, NIAID).



## Evidence for a Non-instructional Model of Thymocyte Differentiation

After development into a DP blast, thymocytes expressing  $\alpha\beta$  TCR have three major fates: about 90% do not receive TCR signals and become small cells with a half-life of 3 days; the remainder either receive a receptor signal leading to rapid apoptotic death [negative selection], or a TCR signal that avoids induction of this death pathway but is adequate for stimulating differentiation towards the CD4 SP or CD8 SP mature phenotype [positive selection]. The MHC restriction of these mature cells correlates with the ability of CD4 to bind to relatively monomorphic regions of class II molecules and of CD8 to bind to monomorphic sites on class I molecules. The differentiation events and signals that determine the MHC class coordination seen on mature T cells between clonotypic T cell receptor specificity and CD4 or CD8 coreceptor specificity remain in dispute. One model proposes that the selective, simultaneous engagement of the TCR and CD4 with class II or the TCR and CD8 with class I on a DP precursor cells “instructs” the cell, by virtue of particular intracellular signal transduction events, to turn off the coreceptor not involved in the recognition event, and to mature along the CD4 or CD8 pathway, respectively. In this model, lineage commitment is concomitant with positive selection for maturation. A second model proposes that the DP precursor loses expression of one or the other coreceptor and commits to a particular differentiation pathway independently of the MHC class specificity of its receptor; a subsequent receptor:coreceptor engagement event is postulated to be critical for full development into a mature T cell. This “non-instructional” model thus also gives rise to mature T cells with coordinate TCR and coreceptor class specificity, but it is based on separate lineage commitment and positive selection events. LI scientists recently provide data in support of the latter model based on the appearance of putative CD8-lineage intermediates in mice lacking class I expression. New data have now refined this analysis, revealing a novel pathway of coreceptor modulation in which CD8 is partially lost from all differentiating thymocytes upon recognition of epithelial MHC molecules (class I or class II), then extinguished in those becoming CD4 cells under the influence of TCR-class II recognition events, or upregulated again on those becoming CD8 cells. In combination with a unique dissection of surface TCR phenotype, these data provide a clearer picture of thymocyte development and strengthen the non-instructional hypothesis of thymocyte lineage commitment. Because T cell development is one of the major models for understanding the role of environmental signals in cell lineage choice, these new findings are of substantial basic importance; they also may have bearing on the origin of rare cell populations with cryptic self-reactivity (J. P. M. van Meerwijk and R. N. Germain, Lymphocyte Biology Section, LI, NIAID; E. O’Connell, LCMI, NIAID).

## Changing Ligand Availability and TCR-Coreceptor Levels During Thymocyte Differentiation Determine Cell Fate

Most thymocyte selection models do not specifically take account of the dramatic changes in TCR expression or coreceptor extinction (rising or falling as much as 50 fold during development from the DP to SP stage, respectively) in evaluating the role of ligand in dictating negative or positive selection events. To re-explore this issue, MHC class II-dependent, MMTV-mediated V $\beta$  specific negative selection has been analyzed using the more precise transitional cell phenotyping recently developed in the LI. This has been done in both normal as well as invariant chain-deficient mice that have a 5 fold reduction in surface class II expression. This analysis has shown that V $\beta$ -specific deletion occurs asymmetrically during thymocyte development, with CD4 lineage-committed cells being lost earlier than CD8 lineage cells. This is in accord with the higher TCR and relevant CD4 coreceptor levels on the former. The quantitative implications of these observations in normal mice are reinforced by the results from the invariant chain-deficient mice, which show a shift in deletion to more mature cells in each lineage, in accord with the higher levels of TCR on such cells that compensates for the lower level of class II-dependent MMTV presentation in these mice. These data provide firm support for an avidity



model of thymocyte negative selection. They also emphasize that the effect of a ligand on thymocyte development cannot be determined based on a simple model involving only the DP blast stage and ligands available to the TCR of these cells on the cortical epithelial cells with which they make contact. Rather, quantitative and qualitative changes in peptide-MHC ligand distribution on the variety of cells in the thymic environment will influence developing T cells in accord with the level of TCR and coreceptor expressed at the time of the interaction. Finally, it appears that negative selection can occur throughout thymocyte differentiation, to at least the pre-export stage of SP T cell maturation. These findings bear on the question of when and to what antigens are developing thymocytes tolerized (L-Y. Huang, J. P. M. van Meerwijk, and R. N. Germain, Lymphocyte Biology Section, LI, NIAID; E. Bikoff, Harvard University).

### Participation of Integrins in Thymocyte Development

The integrin superfamily consists of a large number of heterodimeric cell surface molecules capable of interaction with both extracellular matrix components and other cell surface molecules. In addition to their role in the regulation of the migration/homing of mature peripheral myeloid and lymphoid cells, integrins have been shown to play a crucial role in myelopoiesis and lymphopoiesis in the bone marrow and recent studies have suggested that a number of members of the integrin family may play a role in thymocyte development/differentiation. Previous studies in the LI showed that thymocytes adherent to fibronectin were enriched in DP cells with an immature phenotype (CD3<sup>lo</sup>, CD69<sup>lo</sup>), while the nonadherent population was enriched for more mature "transitional" cells (CD3<sup>hi</sup>, CD69<sup>hi</sup>); the adherence was mediated by  $\alpha 4\beta 1$  which appeared to be constitutively active. In contrast, the capacity of thymocytes to proliferate to the combination of immobilized anti-CD3 and fibronectin was a property of the nonadherent population and was mediated by  $\alpha 5\beta 1$ . We have used multiparameter flow cytometric methods to more precisely characterize the expression of several members of the integrin superfamily ( $\alpha 3\beta 1$ ,  $\alpha 4\beta 1$ ,  $\alpha 5\beta 1$ ,  $\alpha 6\beta 1$ , and  $\alpha L\beta 2$ ) on different thymocyte subpopulations. The results suggest a pathway of T cell differentiation in the thymus in which the majority of the CD34+CD3-DN cells are  $\alpha 4\beta 1^{\text{hi}} \alpha 5\beta 1^{\text{hi}}$ , the DP cells  $\alpha 4\beta 1^{\text{hi}} \alpha 5\beta 1^{\text{lo/-}}$ , and the most mature SP are  $\alpha 4\beta 1^{\text{lo}} \alpha 5\beta 1^{\text{int}}$ . Thus,  $\alpha 4\beta 1$  interactions with fibronectin or VCAM-1 may modulate the transition for CD34+CD3-CD4-CD8- cells to CD3+/-CD4+CD8+ cells. It is quite possible that  $\alpha 5\beta 1$  may also be involved in some of the early steps of this process or that downregulation of  $\alpha 5\beta 1$  is required for maturation to the DP stage. Similarly, as the constitutive activity of  $\alpha 4\beta 1$  is downregulated, while the expression of  $\alpha 5\beta 1$  is upregulated, at some point in the transition from DP to SP, integrin interactions with their target ligands may also play a role at later stages of thymocyte maturation. To directly examine this model, a previously described procedure in which human thymocytes mature and differentiate in lymphoid-depleted mouse fetal thymic rudiments has been used. Under these conditions, highly purified CD34+ CD3-CD4-CD8- human thymic precursor cells give rise to all normal thymocyte subpopulations. The effects of anti-integrin reagents, inhibitory peptides derived from extracellular matrix proteins, as well as soluble VCAM-1 fusion proteins are being evaluated in this model and in comparable model with mouse thymocytes. These studies will permit evaluation of the contribution of each integrin to the processes of positive and negative selection. These data may provide evidence for a relationship between altered integrin or extracellular matrix functions and disturbed thymocyte maturation / selection in man (C. Mojcik and E. M. Shevach, Cellular Immunology Section, LI, NIAID).

### Possible Role of Purinergic Receptors in Thymocyte Selection

Although most attention has been paid to the role of TCR-signaling itself in determining thymocyte fate, recent studies suggest that endogenous steroids can affect the result of such T cell signaling in the thymus. Now LI scientists have developed evidence suggesting that ambient levels of ATP and purines (adenosine in particular) in the thymus may also play a modulating role in





thymocyte selection events. ATP has been shown to cause elevations in intracellular free  $\text{Ca}^{++}$  among thymocyte subpopulations, and more importantly, to have differing effects on distinct subpopulations, causing the most dramatic rise in CD4 SP and DN cells, with lesser responses from DP and CD8 SP cells. Likewise, in vitro studies of dissociated thymocytes or fetal thymus organ cultures show changes in cell survival upon exposure to ATP or adenosine, with the different subpopulations also being affected in distinct ways by ATP and adenosine. These latter data suggest a differing role for P1 vs. P2 receptors expressed on the various subpopulations. Finally, ATP was able to inhibit thymocyte death from glucocorticoid exposure or TCR signaling, whereas adenosine seem to augment these death-inducing signals. Taken together, these results suggest that signals from the various classes of purinergic receptors are integrated with those from environmental steroids and specific TCR engagement to define the fate of developing and mature T cells. These results emphasize the importance of considering events other than antigen-specific signals in thymocyte selection events, including possible defects in these pathways that may lead to export of potentially autoreactive cells giving rise to disease. They also begin to define the specific types of purinergic receptors expressed by distinct T cells subsets and the effects of signaling through these receptors on the fate and function of these T cells. Finally, they open up the possibility of pharmacologic intervention in T cell development or function via drugs interacting with these differentially active and expressed purinergic receptors. (S. Apasov, M. Koshiba, P. Chen, and M. V. Sitkovsky, Biochemistry and Immunopharmacology Section, LI, NIAID; T. Chused, Transmembrane Signaling Unit, LI, NIAID)

## **B Cell Differentiation and Repertoire Generation**

### **$V_H$ -Framework Allotype-Associated B Cell Positive Selection in the Rabbit Appendix**

Although the rabbit immunoglobulin (Ig) H chain genetic region contains multiple variable ( $V_H$ ) genes, 80 to 95% of the B cells of mature rabbits express immunoglobulin that is derived from the same  $V_H$  gene ( $V_H 1$ ). Studies in the LI have shown that B lymphocytes bearing the  $V_H 1$ -associated  $V_{H\alpha 2}$  allotype are preferentially expanded in the rabbit appendix. New work has now found that in mutant *ali/ali* rabbits lacking a functional  $V_H 1$  gene segment, B lymphocyte development is delayed in this gut-associated lymphoid organ, and more developing B cells undergo apoptotic death. This defect is gradually corrected, apparently by the eventual generation and accumulation of B cells bearing  $V_{H\alpha 2}$ -like structures. These arise through use of gene segments related to the preferred  $V_H 1$  segment. The B cells in the rabbit appendix expressing this allotype marker have higher levels of the bcl-2 proto-oncogene, which is known to protect cells from apoptotic death. Together these data suggest that B lymphocytes developing in the rabbit appendix undergo a form of positive selection, perhaps due to a "superantigen-like" interaction of surface immunoglobulin receptors bearing the  $V_{H\alpha 2}$  allotype with an as yet unidentified ligand. Evidence has also been obtained for early and progressive B cell development in the appendix itself, based on the presence of distinct B cells subpopulations with differential expression of the CD43 antigen and surface IgM. Cells with the CD43+/sIgM- and CD43-/sIgM-, but not the CD43-/sIgM+ phenotype express the RAG-1 gene, which is required during early B cell differentiation for effective immunoglobulin heavy and light chain gene rearrangement. These data provide new insight into the origin of B lymphocyte diversity and the role of gut-associated lymphoid tissues in the generation of the mature B cell repertoire in rabbits, and possibly other mammals (H.-T. Chen, R. Pospisil, P. D. Weinstein, and R. G. Mage, Molecular Immunogenetics Section, LI, NIAID).

### **Molecular Genetics of Rabbit Immunoglobulin Gene Rearrangement and Diversification**

To examine the molecular events in generation of a functional and diversified B cell repertoire in the rabbit, DNA circles that result from excision of DNA between rearranging



elements in a common transcriptional orientation were isolated and characterized. Using PCR based methods, it was possible to determine the frequency of rearrangements of  $D_H$  to specific  $J_H$  gene segments.  $J_H2$  and  $J_H4$  rearrangements were most frequent, and the latter became overrepresented in the functional Ig of B cells as a result of post-rearrangement selection. Sequencing of immunoglobulin genes from B cells in the rabbit appendix showed that diversification occurred by both gene-conversion-like and somatic mutational mechanisms. A possible donor gene segment was cloned that could, via gene-conversion, participate in the late development of  $V_{H\alpha2}$ -allotype bearing antigen receptors on the B cells of mutant *ali/ali* rabbits lacking the  $V_{H1}$  gene segment. These studies are creating a detailed picture of the molecular events involved in establishment of the rabbit B cell repertoire (M. Fitts, P. D. Weinstein, P. Fuschiotti, H.-T. Chen, and R. G. Mage, Molecular Immunogenetics Section, LI, NIAID).

## **Mature T Cell Signaling, Function, and Apoptosis**

### **TNF-TNF p75 Receptor As Well As Fas-Fas Ligand Interactions Control TCR-Regulated T Cell Death**

TNF and Fas ligand are related proteins that interact with homologous receptors (p55 and p75, or Fas, respectively) expressed on a variety of cell types, including immature and mature T lymphocytes. LI investigators have now shown that both of these ligand-receptor combinations can contribute to T cell receptor-dependent induction of apoptotic cell death. In contrast to T cell cytokine production, these death pathways do not appear to be sensitive to the CD28 costimulation pathway. Among mature T cells, propioid cell death resulting from vigorous T cell receptor signaling during active cell cycling is completely abrogated by reagents that together block both the Fas and TNF pathways, but not upon blocking of either pathway alone. The kinetics of activity of the two pathways, as well as the cell subsets most sensitive to their action, differ for the two ligand-receptor pairs. The p75, but not the p55, TNF receptor is critical for death induction by TNF. This contribution of both pathways to mature T cell death appears to explain the residual capacity of *lpr/lpr* and *gld/gld* T cells to undergo antigen-dependent elimination. These observations may provide enhanced understanding of such autoimmune diseases as systemic lupus erythematosus, because a mouse model of this human disease has a defect in TNF gene regulation that may prevent normal death regulation of autoreactive lymphocytes. Finally, evidence has been obtained that the Fas-Fas ligand pathway can also lead to death of immature thymocytes, but only in the context of T cell receptor engagement. This may provide a clue to the mechanism of negative thymic selection involved in achieving self-tolerance. These various observations, taken together, give new insight into the basic mechanisms involved in regulating lymphocyte clonal expansion and the potential for autoaggressive lymphocyte function, and open new avenues for attempting control of autoimmune disease (S. Boehme, L. Zheng, G. Fisher, T. Yoo, L. Chen, F. Hornung, and M. J. Lenardo, Molecular Development of the Immune System Section, LI, NIAID).

### **Variant T Cell Receptor Ligands Induce A Novel Pattern of Early Signaling Events**

Peptide-MHC molecule ligands of the  $\alpha\beta$  TCR have traditionally been considered to fall into the agonist or non-agonist categories, based on stimulation of typical effector functions such as IL-2 secretion. Recent work by LI investigators and others has unexpectedly shown that ligand complexes closely related to the peptide-MHC combination used to prime a T cell can also have partial agonist or antagonist properties. New studies in the LI have now revealed that these variant ligands are also characterized by a change in the downstream signal transduction events that follows their interaction with the TCR. Among the earliest events induced by TCR binding to typical agonist ligands is the tyrosine phosphorylation of several TCR-associated proteins, most notably CD3 $\epsilon$  and the  $\zeta$  chain, that latter giving rise to two different species of 21 and 23 kD. This phosphorylation is presumably mediated by src-family kinases (*fyn* or *lck*), and leads to the



SH2-mediated recruitment of a syk-family kinase called ZAP-70 to the tyrosine-phosphorylated  $\zeta$  chain. ZAP-70 is in turn phosphorylated and converted into an active kinase. Defects in ZAP-70 are associated with immune deficiency in humans. Analysis of Th1 clones exposed to either partial agonist peptide-MHC class II complexes, or to the combination of an agonist and antagonist ligand, show an altered pattern of these early events. There is a dose-related increase in the p21 form of phospho- $\zeta$  without a concomitant increase in CD3 $\epsilon$  or p23  $\zeta$  phosphorylated species. ZAP-70 associates with the p21 phospho- $\zeta$ , but it does not become phosphorylated, nor enzymatically active. This pattern is not reproduced by using low levels of full agonist at comparable levels of p21  $\zeta$  formation, indicating that the variant ligands have a qualitative, rather than just quantitative effect on TCR signal transduction. These observations provide a substantially new view of TCR-mediated signaling events, and provide additional leads for dissecting the protein molecular events that follow TCR-ligand binding. The association of altered signaling with differential cytokine production and T cell anergy indicates that understanding these events will have significance for attempts to modify autoimmune disease, T cell responses to the self-antigen of tumor cells, and inappropriate responses to pathogenic organisms. This variant signaling pattern may also play a critical role in effective positive thymocyte selection by self-peptide:MHC complexes (J. Madrenas and R. N. Germain, Lymphocyte Biology Section, LI, NIAID; R. Wange, N. Isakov, and L. E. Samelson, CBMB, NICHD).

### Distinct Role of Individual Cytoplasmic ITAM Motifs in T Cell Signaling

A major pathway of intracellular signal transduction involves the ligand-dependent activation of receptor-associated tyrosine kinases, local tyrosine phosphorylation events, and association of downstream signaling components via SH2-interactions with the initial set of tyrosine phosphorylated sites. The T cell receptor-associated  $\zeta$  and CD3  $\delta$ ,  $\epsilon$ , and  $\gamma$  chains all have in their cytoplasmic regions homologous sequence motifs containing two closely spaced tyrosine residues (ITAMs or immunoreceptor tyrosine-based activation motifs). Because studies in other receptors models have shown that SH2 interactions with tyrosine phosphorylated substrates can be affected by the identity of the adjacent amino acids, and because these residues differ among the ITAMs in the TCR-associated proteins, chimeric TAC (IL-2R $\alpha$ )- $\zeta$ ,  $\delta$ ,  $\epsilon$ , or  $\gamma$  molecules have been generated to examine the role of specific ITAMs in mediating signaling for different T cell responses, especially cytokine production versus apoptotic death. Good expression was obtained with a variety of TAC- $\zeta$  constructs containing one or more of the three adjacent ITAMs of this protein, and with TAC- $\epsilon$  chimeras. Remarkably, after transfection of the  $\alpha$ - $\beta$ - thymoma BW5147, cross-linking of the chimeric proteins using anti-TAC monoclonal antibodies gave different effects, depending on the associated ITAM. Chimeras containing  $\zeta$  ITAMs signalled effectively for both IL-2 production and cell death, whereas TAC- $\epsilon$  chimeras signalled for IL-2 induction, but not apoptosis. These data provide the first evidence for a selective role of the different TCR-associated, ITAM-containing proteins in activating distinct downstream effector pathways, and provide a model for further molecular dissection of these pathways. They complement the variant signaling results obtained using altered TCR ligands, and suggest that differential phosphorylation of the various ITAMs upon TCR engagement with such ligands might in part account for the ability of these ligands to evoke specific subsets of T cell effector functions. These data also indicate that selective drug action might be obtained by agents that interfere with the function of one and not another ITAM (B. Combadiere and M. J. Lenardo, Molecular Development of the Immune System Section, LI, NIAID).

### The Role of B7 Costimulation in T Cell Expression of the B Cell Activating Molecule CD40L

Activation of B lymphocytes for the secretion of immunoglobulins of diverse isotype depends on the T cell expression of the CD40L (gp39) molecule, a member of the TNF family of cell-associated cytokines. Defects in this protein in man result in the immunodeficiency disease



termed X-linked hyper-IgM syndrome. CD40L is not expressed by resting T cells, but is upregulated during activation. Using a transgenic mouse model, LI scientists have found that CD40L expression by CD4+ T cells does not occur upon engagement of the TCR by peptide-MHC class II complexes alone, but also requires costimulatory signals. At least one such signal can be provided by the B7.1/ CD28 interaction, based on experiments using transfected fibroblasts. This is not the only relevant pathway, however. CD40 ligand induction by mixed hematopoietic antigen presenting cells cannot be fully blocked by the CTLA4-Ig fusion protein that inactivates the B7.1 and B7.2 dependent pathway, and cells of CD28-deficient mice also show CD40L upregulation upon TCR stimulation. These data indicate that multiple pathways of costimulation play critical roles in inducing a major activator of B cell differentiation and function. Stimulated B cells themselves are especially active in this costimulatory process, and the activation-related function of B cells in CD40L induction provides a tool for the identification of possible novel costimulatory molecules participating in this pathway. These observations provide new insight into the T cell-B cell interactions that underlie humoral immune responses, and may result in the identification of new target structures for immune modulation (L. Ding and E. M. Shevach, Cellular Immunology Section, LI, NIAID).

## **Cytokines and Cytokines Receptors**

### A Source of Early IL-4 Production Contributing to Enhanced IL-4 Secretion and IgE Production

IL-4 is a key cytokine, regulating multiple aspects of immunity. A major effect is on the production of IgE, important in allergic responses, and on the regulation of inflammatory (Th1) responses often involved in autoimmune disease. LI scientists previously demonstrated that T cell production of IL-4 is dependent on IL-4 itself, raising the question of the initial source of this cytokine. New findings have identified a minor subpopulation of CD4+ T cells in C57Bl/6 mice bearing the NK1.1 marker as providing a key early source of this cytokine. These cells depend on the class I-like molecule CD-1 for their development and stimulation. Based on this, mice deficient in class I expression due to a homozygous mutation in the  $\beta 2$  microglobulin gene were examined and found to have markedly reduced numbers of CD4+NK1.1+ cells. These mice also showed a lack of early IL-4 production upon soluble anti-CD3 antibody challenge, and a greatly diminished IgE response to anti-IgD antibody. Provision of CD4+NK1.1+ cells to these mutant mice, along with a source of CD-1+ presenting cells restored these responses. A second strain of mice deficient in IgE responses, SJL, also has few CD4+NK1.1+ T cells, as predicted by this model. Breeding analyses suggest a limited number of genes involved in this deficiency, and attempts to identify and clone this gene are in progress. These data provide new insight into the cellular basis for initiation of high level IL-4 responses, and point to regulation of CD-1 stimulation of CD4+NK1.1+ T cells as a key event in promoting a Th2-biased phenotype. Examination of the events regulating this newly discovered pathway may provide information about the capacity of helminth infections to elicit high IgE responses, and might reveal a role for this mechanism in establishing the atopic state (T. Yoshimoto and W. E. Paul, General Immunology Section, LI, NIAID; A. Bendelac, Princeton Univ.).

### Differential Stability of the Phenotype of IL-4 and $\gamma$ -IFN Producing T Cells

The cytokine production pattern of CD4+ T cells tends to polarize into the Th1 (high  $\gamma$ -IFN producing) and Th2 (high IL-4 producing) phenotypes. This polarization is itself dependent on the cytokine milieu present during T cell receptor signaling of the naive T cell, with the presence of IL-4 promoting future IL-4 production and suppressing  $\gamma$ -IFN production, and the absence of IL-4, and the presence of IL-12, enhancing eventual  $\gamma$ -IFN production. To begin to understand the stability of these polarized T cell cytokine production patterns, cells from mice transgenic for a T cell receptor recognizing a cytochrome c peptide plus I-E<sup>k</sup> were activated in vitro with peptide,





antigen presenting cells, and various cytokines. After two rounds of activation, these cells were parked in syngeneic mice for 1 month, then removed and stimulated with or without a new round of in vitro priming. The results of these studies showed that the IL-4 producing phenotype was quite stable once induced, with IL-4 exposed cells making IL-4 upon repriming even in the absence of added IL-4, and cells primed in the absence of IL-4 unable to be primed for IL-4 production upon reculture. In contrast,  $\gamma$ -IFN production depended to a great extent on the cytokine environment during the in vitro reculture, with the presence or absence of IL-4 being the key parameter. These data indicate that it is the IL-4 level per se, rather than the balance between IL-4 and  $\gamma$ -IFN that determines the cytokine production of primed T cells, and that the control of the two cytokine genes may be independent events. Other recent studies in the LI have identified a gene expressed during IL-4 stimulation of B cells, termed E4BP4, that could act as an IL-4-regulated transcriptional repressor of genes such as the  $\gamma$ -IFN gene, at least in part accounting for the ability of IL-4 to control production of this inflammatory cytokine response. This new view of the cross-regulation of the Th1 and Th2 effector responses could be of importance in designing strategies to selectively augment or inhibit one or the other during vaccination or for the control of immune based-disorders (J. Hu-Li, C. Chu, and W. E. Paul, General Immunology Section, LI, NIAID).

### Signal Transduction by the IL-4 Receptor

The biological effects of IL-4 are mediated through the binding of this cytokine to its high affinity receptor on a variety of cell types. This binding induces the heterodimerization of the IL4R $\alpha$  chain and the common cytokine receptor  $\gamma$  chain, initiating signal transduction characterized by rapid tyrosine phosphorylation of several substrates. LI scientists have now examined the role of the cytoplasmic portion of the IL-4R $\alpha$  chain in detail, to determine the molecular events involved in converting IL-4 binding into signals for growth and differentiation (CD23 expression, C $\epsilon$ 1 transcription, MHC class II expression) events. One major finding is that the portions of the cytoplasmic tail of IL-4R $\alpha$  that control these two sets of responses are distinct. Growth is primarily regulated by the region lying between residues 437 and 557, which contains a key tyrosine at 497 embedded in a sequence with substantial similarity to that found in the insulin receptor (the I4R motif). Mutation of the Y to F abolished the ability of this receptor to phosphorylate its major substrate 4PS/IRS-1 and to induce growth. To examine whether this was a direct effect related to docking of the 4PS/IRS-1 substrate at the Y497 site, use was made of the yeast two-hybrid system. Taking advantage of the similarity of the relevant regions of the insulin and IL-4R $\alpha$  receptors, the kinase domain of the insulin receptor was changed to contain the IL-4R I4R motif and tested with an IRS-1 fusion target. These yeast experiments show clear evidence for interaction of these proteins, dependent on the presence of Y497 in I4R.

A second region was found to play a dominant role in the signaling for differentiation mediated by the IL4R. Residues 557 to 657 were critical in this regard, as were each of the three tyrosines in this region. All three tyrosines had to be eliminated to prevent such signaling. Chimeric receptor studies revealed a possible domain-domain interaction between this differentiation control region and the 439-555 growth signal containing region. The data are consistent with a model in which the two domains interact with each other, inhibiting receptor function; tyrosine phosphorylation disrupts this interaction and makes each region available for interaction with its substrates (4PS/IRS-1 and possibly Shc for the growth region; STAT-6 for the differentiation region). These data provide an increasingly detailed molecular picture of IL-4R based signaling, information that is of both basic interest, and that may identify sites for rationale drug design of inhibitors or augmenters of IL-4 cytokine action (J. Ryan, K. Nelms and W. E. Paul, General Immunology Section, LI, NIAID; A. Keegan, Holland Lab. American Red Cross).



### CD13 Expression Distinguishes Mast Cells from Basophils, Which Differentially Produce IL-13 and IL-4

Previous studies by LI scientists showed that mast cells and basophils not only released acute inflammatory mediators, but were sources of significant cytokine production. Recent studies have identified a monoclonal antibody reacting with a 161 kd glycoprotein expressed on mast cells and not basophils. This antibody has now been found to bind to CD13, a neutral aminopeptidase also expressed by some macrophages. Using this marker, it has been possible to identify two subsets of mast cells, one of which lacks expression of the high affinity receptor for IgE (FcεR1) due to an absence of γ chain. The properties and functions of this novel subpopulation of mast cells are under investigation. Among the FcεR1+ basophils and mast cells, a striking dichotomy in cytokine production has been recognized. Basophils produce high levels of IL-4 upon cross-linking the FcεR1, or treatment with ionomycin. In contrast, mast cells produce substantial amounts of IL-13, but not IL-4. Although IL-4 and IL-13 are closely related cytokines, their target cells and actions do differ. These new findings may provide clues to allergic and atopic conditions and the roles of basophils and mast cells in these clinical conditions (H.-J. Chen, J. Ryan, C. Kinzer, and W. E. Paul, General Immunology Section, LI, NIAID).

### **Autoimmunity**

#### Fas-Fas Ligand Defects as the Cause of the Human Autoimmune/Lymphoproliferative Syndrome

Scientists in the Laboratory of Clinical Investigation have identified a series of families with children exhibiting an autoimmune/lymphoproliferative syndrome (ALPS) consisting of massive nonmalignant lymphadenopathy, autoimmune phenomena and expanded populations of CD3+, CD4-, CD8- lymphocytes together with antibody-mediated autoimmune disorders. Based on the similarity of some of these disease features to those of *lpr/lpr* or *gld/gld* mice with molecular defects in either Fas or Fas ligand, the structure of these genes were studied in these patients. Several ALPS children were found to have mutations in the apoptosis-inducing molecule Fas that lead to defective Fas-mediated T lymphocyte apoptosis. Transfection studies directly demonstrated that mutant Fas proteins were not only unable to deliver a death signal, but also had a dominant negative phenotype when co-expressed with normal Fas. Family studies showed that the mutations were inherited. This research has thus defined the precise molecular basis of a genetic autoimmune disorder. This new knowledge can be used to develop therapies for this disease. The dominant negative nature of the defects may prove important in understanding the relationship between ligand binding and Fas signal transduction (G. Fisher and M. J. Lenardo, Molecular Development of the Immune System Section, LI, NIAID; W. Strober and S. Straus, LCI, NIAID; J. Puck and F. Rosenberg, NCHGR).

#### Regulation of In Vivo Cytokine Production as a Therapeutic Approach to Autoimmune Disease

Cytokines are major mediators of both immune protection and immune system-dependent diseases such as allergies, asthma, and autoimmunity. Certain autoimmune states appear to involve tissue damage mediated by the Th1-subset of CD4+ T cells. Because of the counter-regulatory or antagonistic action of Th1 and Th2 T cell subsets and their respective cytokine products, deviation the immune response during active Th1-dependent disease towards the Th2 phenotype is an attractive therapeutic possibility. This has been attempted by coadministering IL-4 together with primed, pathogenic autoantigen-reactive T cells in a mouse model of multiple sclerosis termed experimental allergic encephalomyelitis. This approach led to the amelioration of disease. These therapeutic effects correlated with the development of Th2-like, IL-4 producing autoantigen-specific T cells in the host animal. Surprisingly, the production of Th1 cytokines, especially γIFN, was not reduced, despite the amelioration of disease. It appears that rather than



converting existing inflammatory Th1 cells to Th2 cells, or blocking the function of these cells directly, the balance of overall cytokine production, especially the  $\gamma$ IFN:IL-4 ratio, may determine the disease state. These results suggest that *in vivo* manipulation of T cell cytokine production is possible, and provide starting points for the further development of strategies that modulate cytokine expression to reduce allergic or autoimmune diseases. In particular, the ability of newly activated Th2 autoreactive cells to interfere with disease caused by existing Th1 cells, without requiring elimination of the latter, is an encouraging observation with regard to this approach to immunotherapy of autoimmune disease (B. Segal, L. Sakimura, and E. M. Shevach, Cellular Immunology Section, LI, NIAID; M. Racke, Neuroimmunology Branch, NINDS; C. Raine, Div. of Neuropathology, Albert Einstein College of Medicine).

### Thymic Export of Autoreactive Cells

The generation of the primary T cell receptor repertoire by gene rearrangement is an antigen-independent process, and inevitably leads to the production of precursor thymocytes expressing some autoreactive receptors. A primary site of elimination of these potentially harmful cells is in the thymus itself, as a result of antigen-dependent negative selection. Nevertheless, autoimmunity does occur and involves T cells that have developed in the thymus. How the process of generation and elimination of self-reactive T cells in the thymus relates to autoimmune disease remains unclear. Several laboratories have demonstrated that neonatal thymectomy prior to day 3 of life results in a variable but significant incidence of multiorgan autoimmunity disease. LI investigators have now found that the T cells exported during this short time window have a phenotype and reactivity in *in vitro* culture consistent with their polyclonal activation by self-antigens (auto- or SMLR reactive T cells). These results suggest that the autoimmune disease that occurs under these conditions, although showing striking organ selectivity, does not involve only rare individual T cells specific for a narrow range of tissue-specific autoantigens. Rather, it appears that many widely-expressed self-antigens fail to induce appropriate negative selection among thymocytes developing at this time. Introduction of adult hematopoietic cells, especially B cells, into the developing thymus appears to eliminate the development of these SMLR-reactive cells, implying that the survival of these autoaggressive cells occurs as consequence of an imbalance between the rates of production of new thymocytes and the development of a suitable population of class II MHC-positive, negatively-selecting antigen presenting cells in the thymus. These results provide new understanding of how autoreactive cells may escape into the periphery where they can lead to disease. They also indicate that organ-specific disease may arise from the cooperation of these SMLR cells with a smaller number of T cells specific for tissue-specific antigens that also escape thymic elimination due to an absence of these antigens in the thymus. Further characterization of these autoreactive cells and these possible interactions may yield new avenues for intervening in autoimmune processes (E. Payer and E. M. Shevach, Cellular Immunology Section, LI, NIAID; A. Cheever, LPD, NIAID).

### **TCR Recognition Events, MHC Molecule Structure-Function, and Antigen Processing**

#### Relationship Between TCR Binding Affinity for and T Cell Activation by Peptide-MHC Class I Complexes

Short peptides bound to MHC class I molecules are the ligands for  $\alpha\beta$  receptors on CD8<sup>+</sup> T lymphocytes. The method of surface plasmon resonance has been adapted for use in measurement of real time interactions between immobilized  $\alpha\beta$ TCR and soluble MHC class I molecules bound to various synthetic peptides. Using this approach, it has been possible to determine the effective rates of binding to and dissociation from TCR, of various class I-peptide complexes, and to compare these measurements with the ability of the same ligands to activate T cells bearing the



same TCR. For most examples of class I molecules bound to peptides differing from the original stimulatory peptide at putative TCR contact sites, a loss of measurable TCR - peptide/MHC binding using the surface plasmon resonance method was matched by a loss of the ability of the complex to activate the T cell. However, one peptide-MHC class I complex showed no detectable binding to the cognate T cell receptor, yet it was able to activate the T cell, although with lower efficiency than the original ligand. These data provide more extensive quantitative data on the interaction of TCR with their complex ligands, as well as information about the details of molecular structure affecting this binding reaction. They also suggest that simple affinity considerations are inadequate to describe the relationship between ligand structure and biological potency (M. Jelonek, M.P. Corr, L. Boyd, C. Hoes, and D. H. Margulies, Molecular Biology Section, LI, NIAID; A. Bothwell, Yale University).

### Ectophosphorylation and Dephosphorylation Events in the Regulation of T Cell Function

Although tyrosine phosphorylation is a well-recognized regulator of intracellular functions, it is less clear whether comparable phosphorylation and dephosphorylation events involving the ectodomains of surface proteins occur, and if they do, what role they play in regulating cellular activities. Evidence has now been accumulated for such ectodomain phosphorylation and dephosphorylation events, and a possible role for a casein kinase II-like kinase and PP2a phosphatase in these biochemical changes uncovered. Although these studies are still at an early stage, intriguing data have been acquired for tyrosine phosphorylation of the ectodomain of a GPI-linked form of a TCR. This is the same TCR that has been used with the surface plasmon resonance technique to measure the affinity of interaction with peptide-MHC class I complexes, and this experimental model should allow a direct examination of whether tyrosine phosphorylation of this TCR affects its interaction with physiological ligand. Further studies in this and related systems, for example, involving integrins, should determine whether regulated phosphorylation of protein ectodomains plays a modulating role in the activity of surface proteins, and thus, serves as a novel pathway for controlling cell function (S. Apasov, P. Smith, L. Cheng, S. Huang, and M. V. Sitkovsky, Biochemistry and Immunopharmacology Section, LI, NIAID; D. H. Margulies, Molecular Biology Section, LI, NIAID).

### Structural and Kinetic Analysis of MHC Class I-Peptide Interactions and Single Chain Class I Molecules

Surface expressed MHC class I molecules are trimers of MHC-encoded class I heavy chains, non-covalently associated  $\beta 2$  microglobulin, and short peptides. Using the same surface plasmon resonance method employed to study TCR interactions with pre-formed peptide-MHC class I complexes, the formation of these ligand complexes themselves has been investigated. Qualitative information on the requirements for class I-peptide binding has been obtained using a novel method involving systematic cysteine replacement at each position in the peptide and sulfhydryl chemistry to couple the peptide by this residue to the sensor surface. Quantitative data have also been generated by this approach, which reveal that peptide binding is slower than a typical diffusion limited reaction. Preliminary data indicate this may relate to the need for the class I binding site to "accommodate" to its ligand. For experimental and therapeutic purposes, a single chain class I- $\beta 2$ m protein retaining sufficient flexibility for peptide binding would be highly desirable. Such a molecule has been engineered and expressed both on cell surfaces and in bacteria. These molecules can be loaded with specific peptide, after which they act as effective ligands for T cell receptor-dependent lymphocyte activation. These molecules are being used to produce cells expressing only a single functional class I molecule, by introducing a single chain class I- $\beta 2$ m molecule into the  $\beta 2$ m  $\beta$ -background. In this environment, all other class I heavy chains fail to be effectively expressed due to the lack of  $\beta 2$ m expression in *trans*. The *cis*-associated heavy chain in the single chain construct, however, can still be properly folded and





occupied with peptides. Transgenic mice are being generated using the same gene construct. Founder mice have been produced and will be examined for transgene expression. These animals should provide new insight into class I-dependent T cell development, as well as NK and CD8 T cell function (S. Khilko, L. Boyd., S. Sakuma, R. Hunziker, A. D. Plaksin, and D. H. Margulies, Molecular Biology Section, LI, NIAID; L. Lee and M. Mage, LB, NCI).

#### Production, Structure, and Function of TCR V $\alpha$ Domains

Although the detailed structures of the MHC molecule ligands of  $\alpha\beta$  T cell receptors are now known from crystallographic studies, this is not true for the receptor itself. To obtain these data, either complete or partial receptors must be produced in adequate amount and the proper chemical form for crystallization and analysis. It has proved extremely difficult to generate recombinant TCR proteins in large amounts and native conformation. LI scientists have now developed a novel approach involving production of isolated V $\alpha$  domains in bacteria that shows substantial promise in providing at least partial information about TCR structure. Cloned V $\alpha$  gene segments are placed in bacterial expression vectors and after transformation and induction, the expressed protein forms inclusion bodies. These inclusion bodies are solubilized and the resultant protein carefully refolded under empirically determined conditions. In at least one case, this has resulted in a very large amount of soluble, monomeric material reactive with antibodies to the native V $\alpha$ 2 structure. This protein has been used to generate crystals suitable for diffraction, and the collected data, with a resolution to 2.8Å, are currently being analyzed by molecular replacement methods. This approach should yield the molecular structure of at least the V domain of a typical T cell receptor. The same material has been tested for its ability to block activation of the T cell from which it was cloned, and has shown activity in this assay. The data suggest that even in the absence of the TCR  $\beta$  chain, the V $\alpha$  segment has substantial capacity to recognize a peptide-MHC molecule ligand. Extensions of this approach should yield new understanding of the relationship between TCR structure and function (A. Plaksin and D. H. Margulies, Molecular Biology Section, LI, NIAID; S. Chacko and E. Padlan, LB, NIDDK).

#### Invariant Chain Function, MHC Class II Trafficking, and Exogenous Antigen Presentation

MHC class II  $\alpha$  and  $\beta$  chains typically assemble with the non-MHC encoded type II glycoprotein invariant chain shortly after cotranslational import into the endoplasmic reticulum (ER). Using a combination of gene targeted mice and in vitro models, LI scientists have now revealed how invariant chain contributes to assembly and secretory pathway trafficking of MHC class II molecules. These functions depend on a short, antigenic peptide-length region in the middle of invariant chain (termed CLIP) that provides a temporary ligand for the class II binding site, helping to stabilize and properly fold the heterodimer, while at the same time inhibiting binding of either antigenic peptides, or more importantly, peptide-sized stretches of amino acids in the folding intermediates of the large number of proteins in the ER. The precise contribution of CLIP varies with the MHC class II allele, as expected due to the polymorphism of class II molecules. Other regions of invariant chain have now been shown to augment CLIP-driven binding, to provide useful association of the monomorphic invariant chain molecule with diverse class II alleles. Cell fractionation studies have shown the post-secretory pathway of class II-invariant chain trafficking involves movement through early endosomes and late endosomes, to a lysosome-related subcompartment termed MIIC. Invariant chain is removed in proportion to the proteolytic properties of these compartments and the extent of their acidification, and also as a function of time. In the presence of the protein antigen hen egg lysozyme, evidence of processed antigen loading of class II can be found in all these endosomal fractions. However, the extent and the kinetics of this process differ in the three fractions, consistent with a model in which invariant chain removal is a precursor to peptide loading, and in which lack of early invariant chain removal results in the accumulation of the associated class II in the lysosome-like compartment. These



results provide significant new insight into the class II antigen presentation pathway, as well as an intriguing model system for studying protein folding and chaperon interactions. They indicate that differences in invariant chain interaction with various MHC alleles may affect the spectrum of antigenic determinants presented by class II. These data also suggest that different antigenic determinants may be processed and captured by class II in distinct endosomal compartments, and that variation in the rate of intercompartmental transit and proteolytic activity may give rise to distinct antigen presentation capacities in various subpopulations of class II expressing cells, such as B cells, macrophages, and dendritic cells. This knowledge has implications for the cell biology of intracellular protein trafficking, for understanding immunodominance, and for developing more effective means of loading class II molecules with vaccine constituents (F. Castellino, P. Romagnoli, R. Han, G. Zhong, and R. N. Germain, Lymphocyte Biology Section, LI, NIAID; M. Marks, C. Bonnerot, and J. Bonifacio, CBMB, NICHD; E. Bikoff, Harvard University).

### Two Sites for CD4 Binding to MHC Class II Provide Evidence for Ordered Oligomerization as a Key Feature of T Cell Signal Transduction

Most receptors containing or associated with tyrosine kinases are activated by oligomerization. For many growth factor receptors, the growth factors are either dimers or contain two binding regions in a single molecule, accounting for receptor dimers can be formed upon ligand exposure. Both the peptide-MHC molecule ligands of the  $\alpha\beta$  TCR and the receptor itself are monomeric, leaving open the question what types of protein associations or oligomerization events are involved in activating the TCR- or CD4/8 coreceptor-associated src-kinases. Previous work revealed a major binding site for the CD4 coreceptor in the  $\beta 2$  domain of MHC class II molecules. New LI studies now have localized a second site on the MHC class II molecule that regulates the function of CD4 during antigen-induced T cell activation. Remarkably, this second site is on a different chain of the class II heterodimer from that originally identified, lying in the membrane proximal  $\alpha 2$  domain of the  $\alpha$  chain. Molecular modeling indicates that a single CD4 molecule cannot simultaneously contact both the  $\alpha 2$  and  $\beta 2$  sites. Because both class II sites are involved in CD4 function, this indicates that some dimerization event must take place for effective signal transduction. One suitable model involves the binding of two CD4 molecules to a single class II molecule. This would result in dimerization of the *lck*-associated CD4 coreceptor with a single peptide-MHC specific TCR during immune recognition, and could account for activation of this key kinase. Intriguingly, however, the two distant sites in a single class II molecule lie together in the class II "dimer of dimers" seen in HLA-DR1 crystals. Thus, an alternative model is that CD4 binds to a transiently formed complex of two class II ligands and two TCR, stabilizing this dimeric receptor complex and promoting signal transduction. In either case, these findings provide the first evidence for a precise molecular organization of TCR/coreceptor protein dimers during physiologic signaling of T cells, and suggests that "ordered oligomerization" via numerous low affinity contact regions may account for the production of stable complexes suitable for tyrosine kinase activation and TCR -dependent signal transduction. Beyond its implications for a basic understanding of this fundamental immune process, the identification of discrete sites for CD4-class II interactions provides information useful for the development of site-specific drugs that interfere with this recognition and signaling process (R. N. Germain, Lymphocyte Biology Section, LI, NIAID; R. König, UT Galveston).

## **Tumors and AIDS**

### Role of Ly-6 Antigens in the Enhancement of Tumor Growth and Metastasis

Ly-6 antigens are cell-surface molecules anchored in the membrane by GPI-tails rather than hydrophobic polypeptide transmembrane regions. The function of these molecules is unknown, although artificial cross-linking on T cell can lead to cell activation. Recent collaborative studies



have now shown a correlation between Ly-6 expression and the aggressiveness of several different tumors. Cells with enhanced tumorigenicity in a polyoma-induced 3T3 model or a chemical induced adenocarcinoma model show higher Ly-6 levels, and selection for low Ly-6 expression results in cell populations with slower growth and less metastatic tendency. These phenotypic observations suggest a possible participation of Ly-6 in the growth and metastatic potential of transformed cells. This will be directly assessed by various interventions (antisense inhibition, active or passive immunization to provide blocking antibodies, administration of Ly-6 soluble fusion proteins) that should interfere with Ly-6 function either intracellularly or during extracellular engagement of a counter-receptor. These studies will determine whether such intervention can affect the rate of tumor growth or its tendency to disseminate, and may reveal a new parameter useful in grading malignancies, or novel approaches to cancer therapy (P. Korty and E. M. Shevach, Cellular Immunology Section, LI, NIAID; I. P. Witz, Univ. of Tel Aviv).

### High Molecular Weight Thrombospondin Peptide Conjugates That Inhibit Tumor and Endothelial Cell Growth

Many important biological activities involve small regions of larger proteins that can be mimicked by synthetic peptides equivalents. Unfortunately, *in vivo* use of such free peptides as drugs is constrained by their rapid clearance/destruction, and by the limited biological activity of unconstrained, monomeric peptides. One such peptide corresponds to an 18-residue segment in the type 1 repeat of human thrombospondin-1, which shows an ability to block the growth of human melanoma, breast carcinoma, and normal epithelial cell lines *in vitro*, and to inhibit AIDS Kaposi's sarcoma cell and breast carcinoma cell growth *in vivo*. Recent studies have now shown that retro-inverso analogs (all D-amino acids with peptide bond direction also reversed) of the native TSP sequence and various substitutional and truncational analogs are as active as the corresponding L-forward sequences, showing that their biological activities depend principally on the side chain groups (in correct orientation) and very little on the peptide backbone structure and terminal charges. The retro-inverso peptides are much less susceptible to destruction by proteolysis, and thus should exhibit somewhat increased biological half lives. Biological half life may be extended further by covalently linking these peptides to a soluble polymer, such as polysucrose (Ficoll™). Testing of numerous peptide analogs and variants has identified three active motifs: a basic, classical heparin-binding motif, KRFK (of the class BBXB, where B is a basic residue, such as Lys or Arg); and two overlapping WSXW motifs (WSHWSPW). TSP and FGF-2 bind to different determinants on heparin. WSXW peptides completely inhibit binding of TSP but not FGF-2. Both the basic BBXB motif linked to at least one WSXW motif (WSHW or WSPW) is required for FGF-2 inhibition. Coupling efficiency to Ficoll™ is high enough to allow final processing by simple dialysis or ultrafiltration, thus obviating a requirement for size-exclusion chromatography that had hitherto been the scale-up bottleneck, and permitting the preparation of the appreciable quantities of conjugates needed for *in vivo* studies. Peptides with heparin-binding IC<sub>50</sub>s in the submicromolar range and their conjugates have been submitted for testing on animal tumor models and *in vitro* anti-angiogenesis assays. These peptide-polysaccharide conjugates may prove to be highly active agents for ultimate use in human therapy (J.K. Inman, Bioorganic Chemistry Section, LI, NIAID; H.C. Krutzsch and D.D. Roberts, LP, NCI).

### Immune Responses to *Brucella abortus* Conjugated with HIV-1 Envelope Proteins or V3-Loop Peptide Analogs

The ability to bypass the CD4 T cell defect in patients infected with HIV might provide an opportunity for useful therapeutic vaccination against both the virus and organisms responsible for opportunistic infections. Heat-killed *Brucella abortus* is well-known as a T independent type-1 antigen able to stimulate B cell responses without classical CD4<sup>+</sup> T cell help. Studies have now shown that antigenic determinants (peptides) conjugated to this organism can elicit both humoral



and cell-mediated immune responses, include CD8<sup>+</sup> cytotoxic T cells. The antibodies produced to the V3 loop determinant can block syncytium formation by HIV in vitro, and the elicited CTL can kill target cells expressing HIV gp120 as an endogenous protein, showing their ability to recognize naturally processed antigens. CTL generation occurs in CD4<sup>-</sup> mice, indicating that the CD8 response is independent of conventional T cell help when stimulated with this novel antigen formulation. These data suggest a possible route for generation of vaccines effective in neonates or HIV-infected individuals with deficient CD4 T cell responses (J. K. Inman, Bioorganic Chemistry Section, LI, NIAID; B. Golding and H. Golding, FDA).





## Honors, Awards and Scientific Recognition

Laboratory of Immunology scientists play important roles in the U.S. and international scientific communities. They serve on editorial boards of many scholarly publications, on the advisory and scientific review boards of major organizations, act as organizers and major participants in international and national scientific meetings, provide expert advice to numerous biotechnology and pharmaceutical organizations, and give invited lectures about their research at universities and research institutes across the United States and throughout the world.

Dr. William Paul is the editor of the *Annual Review of Immunology* and of the advanced textbook *Fundamental Immunology*, the third edition of which was recently published. He is an advisory editor of the *Journal of Experimental Medicine*, an associate editor of *Cell* and an editorial board member of *Immunity* and *Immunological Reviews*. He is also a transmitting editor of *International Immunology*.

Dr. Ethan Shevach serves on the editorial boards of the *Journal of Immunological Methods*, *Cellular Immunology*, and the *Journal of Biomedical Science*. He is section editor for the immunology section of *Life Sciences* and is an editor of *Current Protocols in Immunology*.

Dr. Ronald Germain is an advisory editor of the *Journal of Experimental Medicine* and a member of the editorial boards of *Immunity* and the *Scandinavian Journal of Immunology*.

Dr. Rose Mage is a member of the editorial board of *Immunogenetics*. Dr. David Margulies is an editor of *Current Protocols in Immunology* and a Section Editor for the *Journal of Immunology*. Dr. Michail Sitkovsky is an associate editor of the *Journal of Immunology*. Dr. Lenardo is a member of the editorial board of *Molecular and Cellular Biology* and an advisory editor of the *Journal of Experimental Medicine*. Dr. John Inman is a member of the editorial board of *Analytical Biochemistry* and is an advisory editor of *Molecular Immunology*.

Dr. Paul is a member of the Medical Advisory Board of the Howard Hughes Medical Institute and of the Advisory Committee of the Department of Molecular Biology, Princeton University. He also is a jury member for the Albert Lasker Medical Research Awards and an Assembly member for the General Motors Cancer Research Award.

Dr. Shevach is vice-chairman of the fellowship review subcommittee of the American Heart Association and a member of the Steering Committee on Transdisease Vaccinology, Global Program for Vaccines, WHO.

Dr. Germain is a member of the Scientific Advisory Boards, Ruggiero Ceppellini Advanced School of Immunology (Naples, Italy), Roche Milano Ricerche (Milan, Italy), and a consultant to the biotechnology companies MedImmune, Inc., Genetics Institute, Inc., Anergen, Inc., and Cell Genesys, Inc. He is also a member of the Human STD Challenge Study Advisory group (NIAID, NIH) and the NIH Inter-Institute Immunology Interest Group steering committee. He recently served on the selection committee for the NIA Scientific Director at NIH.

Dr. Lenardo serves as a member of the steering committee of the NIH Inter-Institute Immunology Interest Group, and was co-chair of "Programmed Cell Death and The Immunology of Aging", sponsored by the NIAID/NIA Task force on Aging and Immunology. Dr. Rose Mage serves as adjunct professor for the George Washington Univ. Genetics Program. Dr. David Margulies is a member of the Scientific Advisory Board of the National Multiple Sclerosis Foundation and the Publications Committee of the American Association of Immunologists.



Dr. Paul was the Sulkin Lecturer at the Univ. of Texas Southwestern Medical Center. He gave the Keynote lecture at the International Conference on Clinical Immunology and the closing lecture at the 9th International Congress of Immunology, both in San Francisco.

Dr. Germain was an invited lecturer at St. Jude's Children's Hospital in Memphis, TN, in the UCSF Immunology Seminar Series, San Francisco, in the Charles Gould Easton Seminar Series, Univ. of Toronto, at Genentech, Inc., at Neurocrine, Inc., and at the European Community Biotechnology Programme Meeting on Basic Immunology of Vaccination. He presented invited plenary lectures at the Second National Conference on Human Retroviruses and Related Infections, the Keystone Symposium "Dendritic Cells: Antigen Presenting Cells of T and B Lymphocytes" and the 9th International Congress of Immunology, where he also was chairman of the symposium on "MHC Class II Antigen Presentation". He spoke at a symposium honoring Baruj Benacerraf, entitled "IR Genes and the MHC: Perspectives for the 21st Century", and lectured at the Institute for Molecular Medicine, Oxford, England and for the "Antibody Club", the London, England city-wide immunology association. He also presented a lecture at the NIH Research Day Festival in the Protein Trafficking Workshop.

Dr. Lenardo chaired a session of and spoke at the Fogarty International Center Conference on Programmed Cell Death, was co-chair of the NIH Festival Symposium "Apoptosis and the Cell Cycle", and lectured in the symposium "Signal Transduction in Differentiation and Transdifferentiation", European School of Molecular Medicine, Brescia, Italy, at the International Union of Biochemistry Symposium on Molecular Recognition, Singapore, and in the NIH Director's Seminar Series. He also presented lectures at the Dept. of Microbiology, UT Galveston, Laboratory of Gene Expression, Istituto Scientifico H. san Raffaele, Milan, Italy, Dept. of Molecular Biology, Univ. of Rome, Italy, Dept. of Pathology, Washington Univ., St. Louis, Searle/Monsanto, St. Louis, Massachusetts General Hospital Cancer Center, Boston, Dept. of Immunology, Univ. of Virginia, and College of P and S, Columbia Univ., New York.

Dr. Margulies was an invited lecturer at the 16th International Congress of Biochemistry, New Delhi, India, the BiaCore Symposium, the Johns Hopkins Immunology Seminar Series, and the Memorial Sloan-Kettering Seminar Series. He served as a workshop chairman at the 9th International Congress of Immunology.

Dr. Shevach was a workshop chairman at the 9th International Congress of Immunology.

Dr. Mage lectured in the Johns Hopkins Seminar Series and the NIH-wide Immunology Interest Group Seminar Series, as well as in the FAES Advanced Immunology Course.

Dr. Gu spoke at the Beth Israel Hospital, Harvard Medical School, Boston, and in several of the NIH-wide Interest groups series.



## **Administrative, Organizational and Other Changes**

The Laboratory of Immunology is a major training center for young immunologists. During the past year, several scientists completed pre- or post-doctoral training periods in the Laboratory. Among these are Matthew Fitts, Galen Fisher, Patrizia Fuschiotti, Ricai Han, Kelley Joe, Achsah Keegan, Edward Levine, Joaquin Madrenas, Paul McGovern, and Tomohiro Yoshimoto. Each of these individuals made important contributions to the Laboratory of Immunology research program. It is anticipated that they will have very productive research careers and that many will attain leadership positions in modern immunology or a related field.

During the past year, several scientists joined the Laboratory of Immunology as postdoctoral fellows or as students. They include Liping Chen, Eileen Farnon, James Hardwick, Russell Hayman, Felicita Hornung, Hua Huang, Huang Huang, Steve Huang, Yasushi Itoh, Michele Johnson, Hidefumi Kojima, Lisa McReynolds, Masao Murakami, Mayumi Naramura, Kannan Natarajan, Clara Pelfrey, and Li Ming Yang. It is expected that they will continue the tradition of excellence established by a long series of trainees in the Laboratory.

During the past year, Dr. Michael Lenardo was granted tenure and made chief of the Molecular Development of the Immune System Section in the Laboratory of Immunology.

Dr. William Paul, Chief, LI, was promoted to Assistant Surgeon General, USPHS.

Dr. Hua Gu, a newly recruited tenure-track appointee, established his own research program in lymphocyte development and began supervising the operation of the NIAID Gene Targeting Facility.

Finally, the Laboratory of Immunology saw the retirement of a very able technician, Mr. Charles Hoes, after 38 years government service, the last 35 of which have been spent in the LI.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00030-27 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Antigen Recognition and Activation of Immunocompetent Cells**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	William E. Paul	Laboratory Chief	LI, NIAID
Others:	John Ryan	Special Volunteer	LI, NIAID
	S.Z. Ben-Sasson	Visiting Scientist	LI, NIAID
	Hang-Jiong Chen	Visiting Fellow	LI, NIAID

COOPERATING UNITS (if any)

**Hebrew University Hadassah Medical Center (S. Z. Ben-Sasson); Jerome Holland Laboratory, American Red Cross (A. Keegan)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**General Immunology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**2.35**

PROFESSIONAL:

**1.35**

OTHER:

**1.0**

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects      •(b) Human tissues      X      •(c) Neither  
 •(a1) Minors  
 •(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A hamster monoclonal antibody has been developed that is specific for a 161 kDa protein expressed on mouse mast cells and some activated macrophages. The protein is not found on most other mature hematopoietic cells and thus can be used to distinguish mast cells from basophils. Among FcεR+ cells, it is generally co-expressed with c-kit. p161 has been purified to homogeneity and sequences of several proteolytic peptides have been obtained. These sequences reveal a high degree of homology with human and rat CD13. Using a rat cDNA clone for CD13, a full-length mouse CD13 clone has been obtained. Stable transfectants expressing mouse CD13 are K-1 positive verifying that CD-13 is p161. CD13 is a neutral aminopeptidase, raising the possibility that it has an important role in mast cell function. The availability of this antibody has made possible the identification of a set of mast cells that lack FcεRI. These cells can be grown in long-term culture in IL-3; they contain histamine, express FcγRII and have Alcian blue positive granules. They express mRNA for the FcεRI α and β chains but lack γ chain mRNA. Transfection of γ chain into FcεRI<sup>neg</sup> mast cells allows the expression of FcεRI. The expressed receptor is functional in that cross-linking it leads to secretion of IL-3. Expression of CD13 on FcεRI<sup>pos</sup> cells allows a discrimination of mast cells and basophils. Short-term-cultured basophils produce IL-4 in response to cross-linkage of FcεRI, but little IL-13 mRNA. By contrast, cultured mast cells are excellent IL-13 producers, but secrete little IL-4. Thus, the two major FcεRI<sup>pos</sup> cells preferentially secrete distinct cytokines, whose actions, while overlapping, tend to affect different cell types. Thus, these cells may prove to have unique roles in mediating allergic inflammatory responses.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00035-20 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Design and Synthesis of Immunomodulators, Vaccine Constructs and Biological Response Modifiers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John K. Inman Section Chief LI, NIAID  
Others: Patricia F. Highet Technician LI, NIAID

COOPERATING UNITS (if any)

Lab. Pathology, NCI, NIH (D. D. Roberts, H. C. Krutzsch); Food and Drug Admin. (B. Golding, H. Golding); Utrecht University Medical School (H. Snippe); Mucosal Immunity Section, LCI, NIAID (R. O. Ehrhardt, W. Strober)

LAB/BRANCH

Laboratory of Immunology

SECTION

Bioorganic Chemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects •(b) Human tissues X •(c) Neither  
•(a1) Minors  
•(a2) Interviews

SUMMARY OF WORK (Use standard unraduced type. Do not exceed the space provided.)

This project is directed toward the design and synthesis of immunogens, vaccine constructs, immunomodulators and biological response modifiers for collaborative studies of both basic and applied nature. Much of this work has been focused upon (1) studying immune responses to *Brucella abortus* conjugated with gp 120 or V3-loop peptides derived from human immunodeficiency virus (HIV-1) strains, with the goal of developing a therapeutic vaccine, (2) developing novel polymer-peptide conjugates that inhibit tumor and endothelial cell growth with potential for the treatment of disease conditions that depend on neovascularization, such as melanomas, breast carcinoma, Kaposi's sarcoma and diabetic retinopathies. Emphasis has been placed on studying the effects, *in vitro* and *in vivo*, of polyvalent presentation of biologically active molecules (peptides, lipopolysaccharides, ligands, haptens, etc.), covalently linked to soluble high molecular weight polymer or protein carriers. New reagents and methods have been developed in this section for synthesizing novel conjugates used for these studies.

Conjugates of HIV-1 derived peptides covalently linked to heat-inactivated *B. abortus* have been found by collaborative investigators at CBER, FDA to elicit virus neutralizing (syncytia inhibiting) antibodies, predominantly of IgG2a isotype, as well as peptide-specific cytotoxic T cells, even in mice severely depleted of CD4+ helper T cells. These constructs are being actively investigated as potential therapeutic vaccines for treating AIDS patients.

Collaborative studies are being carried out with investigators in the Laboratory of Pathology, NCI, on biological responses to synthetic peptides linked to soluble polymer carriers. The sequences are taken from the extracellular matrix protein, thrombospondin-1. A number of variants of a peptide from the type 1 repeat unit have been synthesized and covalently coupled to highly branched polysucrose (Ficoll™). *In vitro* and *in vivo* experiments have shown that many of these conjugates are potent inhibitors of endothelial and tumor cell growth; and thus, they may be useful in blocking metastatic growth of solid tumors. A US patent has recently been filed based on these findings.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00036-30 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	M. Fitts	IRTA Fellow	LI, NIAID
	H.-T. Chen	Senior Staff Fellow	LI, NIAID
	P. D. Weinstein	Special Volunteer	LI, NIAID
	P. Fuschiotti	Visiting Associate	LI, NIAID
	R. Pospisil	Visiting Fellow	LI, NIAID
	E. Schiaffella	Visiting Fellow	LI, NIAID

COOPERATING UNITS (if any)

ARD, USAMRID, Ft. Detrick, Frederick, MD (A.O. Anderson); Dept. Biol. Sci. Florida State U., Tallahassee, FL. (K. Roux); Dept Tumor Cell Biol. St. Jude Children's Res. Hosp., Memphis, TN (LL.M. Hendershot); Dept Microbiol., Med. Coll. Ohio, Toledo, OH (D.W. Metzger); Spring Valley Labs, Inc. (R. Shaw)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects      •(b) Human tissues    X    •(c) Neither  
     •(a1) Minors  
     •(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We used techniques of immunogenetics and molecular biology to study rabbit immunoglobulins, and other genes including RAG-1 and RAG-2, which are necessary for gene rearrangements to occur during lymphocyte development. We investigated the development of anatomical sites such as appendix follicles and germinal centers in gut-associated lymphoid tissues and the regulated expression and sequence diversification of Ig genes during lymphoid cell development. Whereas B cells with rearranged VH1 predominate in normal rabbits, in homozygous *Alicia* mutant rabbits (*ali/ali*) the VH1 gene is deleted and B cells with upstream VH genes rearrange. We found differences between appendix development in normal and *ali/ali* rabbits based on immunohistochemistry, analyses of cell proliferation and apoptotic death. The development of the appendix in mutants appears to be retarded compared to normals. As populations of B cells bearing VHa2-like epitopes develop in mutant animals, appendix development appears more like normals. A higher proportion of B cells expressing the a2 allotype may receive strong signals to survive rather than undergo apoptosis. VHa2-positive B cells express high levels of Bcl-2 protein compared to a2-negative B cells. This suggests that B cells with FR allotypic motifs may become resistant to programmed cell death via the Bcl-2 pathway. The a2 allotype probably plays functional role(s) in selection and effective expansion of B cells in the appendix. We used the surface markers CD43 and IgM to distinguish two appendix cell populations from 6 to 8-week-old rabbits that expressed RAG-1 transcripts. We speculate that the appearance of CD43 during different stages of B-cell maturation may be related to the function of the appendix as a site of both B-cell development and diversification. IgM associated B cell receptor molecules were found on rabbit B cells as heteromeric structures with nonreduced molecular weights of approximately 75 kDa and 135 kDa composed of 37 and 42 kDa subunits. Immunological studies with monoclonal antibodies suggested that these proteins are the rabbit homologues of murine Ig-β (B29) and Ig-α (mb-1).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00134-33 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard Asofsky Section Chief LI, NIAID  
Others: Ada Brooks Biological Technician LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects •(b) Human tissues X •(c) Neither  
•(a1) Minors  
•(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TH 2.2 is a cloned (B lymphoma X B lymphocyte) somatic cell hybrid line which responds to exposure to bacterial polysaccharide by reduced growth, and by the secretion of the cytokines IL-3, IL-6, and GM-CSF, as well as by increased secretion per cell of IgM above the amount secreted constitutively. Exposure of TH 2.2 to amounts of LPS in doses  $>1\mu\text{g/ml}$ , but not to lower doses renders the cells unresponsive to many of the inductive effects of LPS. The unresponsiveness lasts at least 20 cell generations, but is recovered eventually. Maintenance of unresponsiveness may depend on the presence of fetal calf serum in the growth medium; transfer of unresponsive cells to medium with very low concentrations of serum or to serum-free medium restores responses substantially or completely. Clonal analysis shows that several individual, highly sensitive clones can each be rendered unresponsive by treatment with LPS, and that individual unresponsive clones obtained from pretreated cells usually recover responsiveness. Finally, we have obtained promising results with an "ELISABLOT" assay for secreted products, beginning with IgM. This assay can enumerate secreting cells, and can be adapted to the measurement of the amounts secreted by each cell. These studies aim to understand the functional diversity found in many cell lines when clonal progeny or individual cells are examined. There is evidence that some of this diversification can be modified or regulated. The fact that LPS can nonpermanently modify the response to itself suggests that cell behavior can be modified by treatments relatively remote in time from the stimuli studied. Modulation of resistance to LPS by change of medium also suggests that "background" signalling may be important in determining responses to signals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00224-14 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Receptors, Co-Receptors, and Counter-Receptors for T Cell Activation**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. M. Shevach	Section Head	LI, NIAID
Others:	L. Ding	Visiting Associate	LI, NIAID
	C. Mojcik	Staff Fellow	LI, NIAID
	R. Ortmann	Staff Fellow	LI, NIAID
	V. Rostapshov	Visiting Associate	LI, NIAID

COOPERATING UNITS (if any)

**LMS, NIAID, NIH (J. Coligan); Dept. of Cell Research and Immunology, Tel Aviv, University, Tel Aviv, Israel (Prof. I. P. Witz)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Cellular Immunology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**5.5**

PROFESSIONAL:

**4.5**

OTHER:

**1.0**

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects     
 ☒ (b) Human tissues     
 X     
 ☒ (c) Neither  
     ☐ (a1) Minors  
     ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The interaction of the T cell receptor (TCR) with its specific ligand, peptide-MHC, is only one component of the events required for the activation of antigen-specific T cells. The primary goals of our studies are to characterize cell surface antigens (co-receptors) and their soluble or cell associated ligands (counter-receptors) which play critical roles in cell-cell interaction and in the interaction of T cells with their environment. Our studies have focused in a number of distinct areas: 1) We have examined the accessory cell requirements for induction of CD40L expression on CD4+ T cells and demonstrated that expression of the B7 antigen was both necessary and sufficient for induction of the CD40L. To directly examine whether the B7/CD28 costimulatory pathway is the exclusive pathway for the induction of the CD40L, CD28 deficient mice were stimulated with anti-CD3 in vivo or in vitro. Induction of the CD40L was comparable on T cells from CD28 deficient mice and normal mice. These results demonstrate that costimulatory signals resulting from B7/CD28 interactions as well as a non-B7/CD28 pathway can result in effective induction of CD40L expression. 2) Integrins represent a candidate group of cell surface receptors that may control the homing and population of the thymus by T cell precursors and the subsequent migration of developing thymocytes through the thymic architecture. To further characterize the role of the integrins  $\alpha 4\beta 1$  and  $\alpha 5\beta 1$  in thymocyte differentiation, we correlated the expression of these integrins with other markers of thymocyte differentiation. The earliest T cell precursors in the thymus selectively expressed both  $\alpha 4\beta 1$  and  $\alpha 5\beta 1$  at high levels and that these integrins may play a role in the homing of these cells from the bone marrow to the thymus as well as in the maturation of precursors to CD4+CD8+ thymocytes. 3) A positive correlation was observed between the expression of Ly-6 antigens on several clones of a polyoma virus transformed BALB/c 3T3 cell and the tumorigenicity of these clones. Moreover, tumors that appear following a relatively short latency period tend to express more Ly-6 than tumors appearing after a prolonged latency. These results raise the possibility that an in vivo encounter between an Ly-6 expressing tumor cell and an Ly-6 counter-receptor may confer upon the Ly-6 high expressor the high malignancy phenotype.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00226-14 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	M. Fitts	IRTA Fellow	LI, NIAID
	H.-T. Chen	Senior Staff Fellow	LI, NIAID
	P. D. Weinstein	Special Volunteer	LI, NIAID
	P. Fuschiotti	Visiting Associate	LI, NIAID
	R. Pospisil	Visiting Fellow	LI, NIAID
	E. Schiaffella	Visiting Fellow	LI, NIAID

COOPERATING UNITS (if any)

ARD, USAMRID, Ft. Detrick, Frederick, MD (A.O. Anderson); Dept Biol. Sci. Florida State U., Tallahassee, FL (K. Roux); Dept Tumor Cell Biol. St. Jude Children's Res. Hosp., Memphis, TN (L.M. Hendershot); Dept. Microbiol. Med. Coll. Ohio, Toledo, OH (D.W. Metzger); Spring Valley Labs, Inc. (R. Shaw).

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Molecular Immunogenetics Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**3.5**

PROFESSIONAL:

**2.5**

OTHER:

**1.0**

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We studied genes of the rabbit immune system by techniques of molecular biology and immunology. PCR allowed us to specifically identify excision circles resulting from DH to JH and D $\beta$  to J $\beta$  DNA rearrangements. Extrachromosomal circular DNA purified from rabbit bone marrow cells was assayed by PCR to determine the relative in vivo rearrangement frequencies of Ig DH to JH genes. DH genes rearranged to individual JH genes with different frequencies. This bias did not correlate with potential sequence overlaps in the DH or JH coding sequences. The JH2 and JH4 genes were the preferred targets of recombination in primary rearrangements. Analyses of genomic VDJH indicated that B cells expressing VDJH4 heavy chains survived and dominated in the bone marrow due to post-rearrangement selection. We obtained new data that support the proposal made many years ago that the rabbit appendix could be a bursal equivalent. The DNA sequences of rearranged heavy chain variable region genes from B cells in the light and dark zones of appendix germinal centers from 6-week-old rabbits were highly diversified in CDR2, probably through both gene conversion and somatic hypermutation; some LZ sequences were closer to germline. We derived simple evolutionary trees that showed the relationships of cells with different sequences to each other and to single progenitors. Potential donors that could have participated in gene conversion-like alterations of rearranged genes were identified. We prepared a cosmid library containing 35-45 kb fragments of rabbit genomic DNA in order to further characterize VH gene expression and diversification in the rabbit. We found and characterized an upstream VH gene that may act as a donor for gene conversion-like alterations of rearranged VH-gene sequences that lead to production of VH structures resembling those encoded by a deleted VH1 gene in mutant ali/ali rabbits. We also found and are characterizing germline DH and JH genes. We produced anti-RAG-2 antibodies and used them in studies of developing rabbit thymus and appendix tissues.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00349-12 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Structure and Function of Murine Class II MHC Genes and Gene Products**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	R. Han	Visiting Associate	LI, NIAID
	S. Fleury	Visiting Fellow	LI, NIAID
	A. Fox	Research Technician	LI, NIAID
	A. Rinker, Jr.	Research Technician	LI, NIAID
	L-Y. Huang	Research Technician	LI, NIAID

COOPERATING UNITS (if any)

**Harvard University (D. Wiley); Univ. Texas Galveston (R. Koenig)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Lymphocyte Biology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**3.6**

PROFESSIONAL:

**2.8**

OTHER:

**.8**

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects     
 ☐ (b) Human tissues     
 ☒ (c) Neither  
     ☐ (a1) Minors  
     ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

**Class II major histocompatibility complex (MHC) gene products** play critical roles in a variety of **T and B lymphocyte** responses. Biochemical and functional analyses have been used to investigate the relationship between class II structure and peptide antigen presentation. The kinetics of class II peptide binding have been examined and two distinct phases of binding identified; a rapid initial binding with a rapid off rate, and a slow accumulation of long-lived binary complexes. Low affinity binding was unexpectedly found to preserve class II molecules from denaturation at physiological temperature. These new insights into the biochemical behavior of class II help provide a deeper understanding of how antigen capture by class II molecules occurs under physiological conditions.

In addition to binding peptide and being recognized by clonally distributed T cell receptors, class II molecules participate in T cell selection in the thymus and mature T cell activation in the periphery by interacting with the **CD4** molecule that is also the receptor for HIV-1. We have used site-directed mutagenesis to define the site(s) of interaction of class II molecules with CD4. In addition to our previous identification of a major binding site in the  $\beta 2$  region of the class II molecule, we have now located a second important site in the  $\alpha 2$  domain. These two sites on a single class II heterodimer cannot both bind to an individual CD4 molecule simultaneously. The two sites do lie close together in the recently published crystal structure of the class II "dimer of dimers". Taken together, these results provide the first evidence for organization of higher order oligomeric signal transduction complexes involving the T cell receptor and CD4 coreceptor. These latter findings, in concert with our studies of T cell signal transduction upon exposure to distinct peptide-MHC ligand complexes (see Z01 AI 00403-12 LI), are defining the molecular mechanisms involved in MHC-dependent antigen recognition by, and activation of T lymphocytes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00394-12**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Molecular Genetic Analysis of Lymphocyte Function**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Charles Hoes	Biologist	LI, NIAID
	Rosemarie Hunziker	Sr. Staff Fellow	LI, NIAID
	Marie Jelonek	Medical Staff Fellow	LI, NIAID
	A. Daniel Plaksin	Visiting Fellow	LI, NIAID
	Kannan Natarajan	NRC Fellow	LI, NIAID

COOPERATING UNITS (if any)

**LB, NIDDK (E. Padlan); MB, NCI (J. Berzofsky); LB, NCI (M. Mage, L. Lee); Mt. Sinai School of Medicine (W. Yokoyama); Yale University (A. Bothwell)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Molecular Biology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**6.0**

PROFESSIONAL:

**6.0**

OTHER:

**0**

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects     
 ☐ (b) Human tissues     
 ☒ (c) Neither  
     ☐ (a1) Minors  
     ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of these studies has been on the functional role of the MHC class I molecule and its interactions with T cell receptors. Model systems have been developed for studies of these functions *in vivo* and *in vitro*. Accomplishments during the past year include: 1) quantitative measurement of the interactions between purified T cell receptors and purified MHC class I molecules complexed with a panel of antigenic peptides; 2) engineering of large amounts highly purified T cell receptor domains, and their use for crystallization and functional studies; 3) evaluation of the interaction of soluble H-2D<sup>d</sup> with NK cells bearing the Ly-49 surface protein.

These studies are providing a new quantitative understanding of the binding interactions between MHC class I-peptide complexes and the receptors of T lymphocytes and NK cells. They may also help produce a detailed structure of the T cell receptor. Together, these investigations will help develop a more complete molecular picture of how immune recognition events involving MHC class I molecules contribute to regulation of the functions of these two important effector cell types.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00403-12 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Analysis of T Cell Receptor Structure, Function, and Repertoire**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	J. van Meerwijk	Visiting Fellow	LI, NIAID
	J. Madrenas	Visiting Associate	LI, NIAID
	E. Levine	HHMI Medical Scholar	LI, NIAID
	A. Fox	Research Technician	LI, NIAID
	A. Rinker, Jr.	Research Technician	LI, NIAID
	L-Y. Huang	Research Technician	LI, NIAID
	J. Wang	Summer student	LI, NIAID

COOPERATING UNITS (if any)

**LCMI, NIAID (B. J. Fowlkes); L. Samelson (CBMB, NICHD); Univ. of Naples (L. Racioppi)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Lymphocyte Biology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**3.6**

PROFESSIONAL:

**1.6**

OTHER:

**2.0**

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects     
 ☐ (b) Human tissues     
 ☒ (c) Neither  
     ☐ (a1) Minors  
     ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antigen-specific T lymphocyte activation occurs through the clonally distributed T cell receptor (TCR), which is involved in thymocyte selection and peripheral T cell effector responses. How TCR-dependent lineage commitment from a common precursor occurs for the two major subsets of T cells, the CD4+ and CD8+ cells, remains unknown. Furthermore, the quantitative and qualitative relationships between receptor occupancy and signaling for differentiation are also poorly understood. This project uses cellular, biochemical, and molecular approaches to study differentiation of thymocytes and activation of T cells upon ligand engagement. Our previous studies suggested that thymocyte development could involve lineage choice and partial maturation that is independent of the MHC class involved in T cell recognition, followed by complete maturation (positive selection) of those cells whose remaining expression of CD4 or CD8 matched the MHC class-specificity of the T cell receptor. Studies of gene targeted and chimeric mice support this conclusion and show that recognition of thymic epithelial MHC per se is critical to the first step in development. Characterization of mature and transitional phenotype cells in invariant chain-deficient mice has also allowed new insights into the quantitative parameters of thymocyte deletion.

We previously reported that some TCR ligands evoke only a subset of T cell effector responses, and that such variant ligands can selectively antagonize cytokine production induced by receptor agonists. New studies show that variant ligands induce only a subset of the tyrosine phosphorylation events typically accompanying agonist recognition. These variant signal transduction events indirectly affect biological responses such as anergy, and can arise not only from changes in the TCR ligand, but also alterations in the signaling machinery of the T cell. Along with our work on the molecular organization of T cell signal transduction complexes (Z01 AI 00349-12 LI), this helps explain how protein-protein interactions result in antigen receptor-dependent second messenger generation, and how specific intracellular signals contribute to the downstream gene activation events in T cells that control effector function and tolerance induction.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00425-11 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lymphocyte Physiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. M. Chused Section Head LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Transmembrane Signalling Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects •(b) Human tissues X •(c) Neither  
•(a1) Minors  
•(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential, intracellular free ionized calcium, and intracellular pH, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized. These studies have revealed extensive "feed-forward" and "feed-back" regulatory relationships between ion channel opening, membrane potential, activity of the calcium pump, and rate of phosphatidyl inositol turnover. These mechanisms differ in the T, B and monocyte/granulocyte lineages. The calcium responses mediated by purine receptors are being examined.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00427-11 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity *In Vivo* and *In Vitro***

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	<b>M. V. Sitkovsky</b>	<b>Section Head</b>	<b>LI, NIAID</b>
Others:	<b>S. Apasov</b>	<b>Visiting Associate</b>	<b>LI, NIAID</b>
	<b>M. Koshiba</b>	<b>Visiting Fellow</b>	<b>LI, NIAID</b>
	<b>P. Smith</b>	<b>Research Associate</b>	<b>LI, NIAID</b>
	<b>P. Chen</b>	<b>Research Associate</b>	<b>LI, NIAID</b>

COOPERATING UNITS (if any)

**LI, (Dr.Tom Chused); University of Missouri (Dr.Gary Weisman)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Biochemistry and Immunopharmacology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**3.5**

PROFESSIONAL:

**3.0**

OTHER:

**0.5**

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues    X    ☒ (c) Neither  
     ☒ (a1) Minors  
     ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The antigen-specific cytotoxicity mediated by cytolytic T lymphocytes (CTL) is an important cellular mechanism of antiviral and antitumor immune responses. The ultimate goal of our studies is to reveal the cellular and molecular requirements for CTL effector functions and differentiation of immature CD4+8+ thymocytes into functional CD8+ CTL. We are currently focusing on the functional role of a novel set of cell surface proteins - the P2 (extracellular ATP) and P1 (adenosine) classes of purinergic receptors, which we propose to be intimately involved in CTL generation. According to our hypothesis the signaling through purinergic receptors may interfere with TCR-triggered activation pathways in differentiating thymocytes and may also be part of the apoptotic pathway leading to lymphocyte death. These studies may help us to understand the molecular mechanisms of immune response; additionally, they may provide novel targets for immuno-modulation. ATP<sub>0</sub>-triggered [Ca<sup>++</sup>]<sub>i</sub> increases in different subsets of thymocytes have been observed, providing evidence that thymocytes do indeed express functional purinergic receptors. A quantitative competitive RT-PCR method has been developed to study the expression of P2 receptors and used to show that apoptotic stimuli (steroids, ATP, adenosine) transiently upregulate expression of purinergic receptors. Signaling through purinergic receptors may have dramatic consequences in thymocyte development as evidenced by differential susceptibility of immature DP and SP thymocytes to apoptosis-inducing signals through P1 (adenosine) and P2 (ATP) purinergic receptors in both short-term (4-16 hrs) and long-term (5-7 days) fetal thymus organ culture assays. Signaling through P1 and P2 receptors has been shown to be able to interfere (antagonize or synergize) with TCR- and steroid-triggered apoptosis triggering in thymocytes. Inhibition of protein synthesis (in experiments modeling early apoptotic events in thymocytes) enhances the expression of P2 receptors. These results are compatible with the outcomes predicted by the purinergic receptors model of thymocyte differentiation. Experiments (targeted inactivation of purinergic receptors with antisense mRNA oligos, transfected constructs, and P2x gene knockout mice *in vivo*) are in progress to determine the contribution of individual P2 and P1 receptors to T cell functions and differentiation. These studies are expected to be greatly simplified by use of the *in vitro* differentiating, immortalized thymocyte clones that we established from p53 KO mice.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00493-09 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interleukin-4 (IL-4)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	William E. Paul	Laboratory Chief	LI, NIAID
Others:	Keats Nelms	Special Volunteer	LI, NIAID
	John Ryan	Special Volunteer	LI, NIAID
	Tomohiro Yoshimoto	Visiting Fellow	LI, NIAID
	Charles Chu	Staff Fellow	LI, NIAID
	Hua Huang	Special Volunteer	LI, NIAID
	S.Z. Ben-Sasson	Visiting Scientist	LI, NIAID

COOPERATING UNITS (if any)

Princeton University (A. Bendelac); Jerome Holland Laboratory, American Red Cross (A. Keegan)

LAB/BRANCH

Laboratory of Immunology

SECTION

General Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.45

PROFESSIONAL:

2.45

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      X      ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The differentiation of naive CD4+ T cells into mature T cells that produce either IL-4 or IFN $\gamma$  is controlled by IL-4 itself. If IL-4 is present at the time of priming, T cells develop into IL-4-producers while if it is absent they differentiate into IFN $\gamma$  producers. Using T cells from mice transgenic for genes encoding a T cell receptor specific for a cytochrome c peptide, the stability of cytokine-producing phenotypes was studied. After transgenic T cells were primed in vitro, they were transferred to syngeneic hosts, remained there for one month and then tested, after an in vitro "re-priming", for cytokine-producing phenotype. IL-4-producing capacity was stable but cells that had failed to produce IFN $\gamma$  initially could become IFN $\gamma$ -producing cells if re-primed in the absence of IL-4, implying that the regulation of production of the two cytokines is different and that the presence or absence of IL-4-production is a more reliable measure of the phenotype of activated CD4 cells than is IFN $\gamma$ -production. The source of the IL-4 that acts at the time of initial in vivo priming was sought. A set of CD4+, NK1.1+ T cells specific for CD-1 was shown to promptly produce IL-4 in response to in vivo activation. These cells appear to be diminished in number and function in two strains of mice,  $\beta_2$ -microglobulin knock out and SJL. Both strains of mice have defects in IgE and IL-4 production in response to polyclonal in vivo B cell activation, indicating the importance of CD4+, NK1.1+ T cells as a source of IL-4 at the outset of responses.

The IL-4 receptor transduces signals mediating the function of the cytokine. Growth and differentiation are largely controlled by distinct domains of the cytosolic portion of the receptor. The region between amino acids 437 and 557 contains a region (the I4R motif) with a single tyrosine to which the substrate 4PS docks. This region is essential for IL-4-mediated growth. It also can mediate differentiation (i.e. induction of CD23 and germline C $\epsilon$  transcripts as well as activation of STAT-6), but only to a small extent. By contrast, the region between amino acids 557 and 657 does not mediate phosphorylation of 4PS but induces CD23 and germline C $\epsilon$  very efficiently. It contains three tyrosines; mutation of all 3 tyrosines ablates this activity but if any one of the tyrosines is not mutant, the region can still induce IL-4-mediated differentiation. Preliminary results indicate that the two domains interact and that this interaction results in inhibition of function.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00545-07 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Antigen Processing and Presentation**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	P. Romagnoli	Visiting Associate	LI, NIAID
	G. Zhong	Visiting Fellow	LI, NIAID
	F. Castellino	Visiting Associate	LI, NIAID
	C. Reis e Sousa	Visiting Fellow	LI, NIAID
	L-Y. Huang	Research Technician	LI, NIAID
	A. Fox	Research	

COOPERATING UNITS (if any)

**CBMB, NICHD (M. Marks, J. Bonifacino); Harvard Univ. (E. Bikoff)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Lymphocyte Biology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**4.6**

PROFESSIONAL:

**3.6**

OTHER:

**1.0**

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects     
 ☒ (b) Human tissues     
 X     
 ☒ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

$\alpha\beta$  T cells respond to peptide-major histocompatibility complex (MHC) molecule ligands. How protein antigens are transformed into peptides suitable for such binding and the intracellular pathways followed by MHC molecules both before and after peptide association are critical to understanding T cell immunity. MHC class II molecules assemble in the presence of invariant chain (Ii) and Ii plays important roles in regulating the early trafficking of MHC class II, as well as directing its movement in the endocytic pathway. We have found that association of the ER-resident protein calnexin with Ii prevents Ii degradation, and retains it in this organelle, so that it achieves a high molar ratio to class II and saturates class II binding sites in the endoplasmic reticulum. New studies show that interaction of Ii varies with the allele of class II. This appears to reflect binding site polymorphism affecting association with the CLIP segment of Ii. Other regions of Ii modulate this CLIP-related variation in binding strength, in accord with our modular model of Ii.

Using transfected cells expressing various combinations of wild-type and mutant MHC class II and Ii molecules, we have found that Ii is essential for presentation of only some peptide determinants within a single protein antigen. Signals in the cytoplasmic tails of the MHC class II  $\alpha$  and  $\beta$  chains control Ii-independent presentation of other determinants. These data suggest that two separate pools of MHC class II molecules (newly synthesized and recycling, mature) provide for maximal effective capture of antigenic information. Pulse-chase labelling, immunoprecipitation, and gradient density fractionation of B lymphoblasts show that class II traffics to multiple endocytic compartments, consistent with class II acquisition of different protein determinants in compartments of differing pH and hydrolytic capacity.

Intact extracellular proteins are usually not converted into peptides bound to MHC class I molecules, but some exceptions involving particulate antigen have been reported. We have examined the ability of phagocytic stimuli to promote class I presentation of exogenous soluble protein. Our studies suggest that this class I presentation under these conditions reflects the rare breakdown of the phagosome membrane and entry of the antigen into the conventional cytoplasmic class I processing pathway. These data are of significance for vaccine design, and may also bear on CD8 T cells responses to some pathogens that normally reside in endocytic vesicles, but occasionally escape into the cytoplasm.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00565-06 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Lymphocyte Signalling Pathways Involving NF- $\kappa$ B Regulators and Other Molecules**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	<b>Michael J. Lenardo</b>	<b>Senior Investigator</b>	<b>LI, NIAID</b>
Others:	<b>Matthew Freedman</b>	<b>HHMI Scholar - Univ. Michigan</b>	<b>LI, NIAID</b>
	<b>B. Combadiere</b>	<b>INSERM Fellow</b>	<b>LI, NIAID</b>

COOPERATING UNITS (if any)

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Molecular Development of the Immune System Unit**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**1.5**

PROFESSIONAL:

**1.0**

OTHER:

**0.5**

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects     
 ☐ (b) Human tissues     
 ☒ (c) Neither  
     ☐ (a1) Minors  
     ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Regulation of genes for several lymphokines as well as other molecules involved in the immune response depend on a 10 bp DNA sequence termed  $\kappa$ B. This sequence binds a family of nuclear proteins related to the mammalian Rel oncogene and the drosophila protein, dorsal. Importantly, the  $\kappa$ B sequence is found in the human immunodeficiency virus (HIV). We have established a system to study NF- $\kappa$ B in nontransformed T cell clones following stimulation by antigen and antigen-presenting cells (APCs). Recently we have focused on comparing the regulation of various NF- $\kappa$ B subunits in the T<sub>H</sub>1 and T<sub>H</sub>2 subsets of CD4<sup>+</sup> T lymphocytes. It is believed that there may be a switch from the T<sub>H</sub>1 to the T<sub>H</sub>2 subset in the late course of AIDS. We have therefore been studying the transcriptional activating function of the human immunodeficiency virus long terminal repeat (HIV LTR) which is controlled by NF- $\kappa$ B. Our preliminary findings suggest that the HIV LTR is more active in T<sub>H</sub>2 cells suggesting that viral production might be accelerated in the late phases of AIDS during the T<sub>H</sub>1 to T<sub>H</sub>2 switch. Further work will be directed at confirming and extending these findings and determining how various subunits of the NF- $\kappa$ B family regulate the binding site within the HIV LTR.

One of the most interesting features of antigen signalling in T cells is that the same T cell receptor (TCR) complex can lead to activation, which includes NF- $\kappa$ B induction, or to programmed cell death (apoptosis). The signal pathways that discriminate between these two outcomes are unknown. We have initiated studies to systematically mutagenize the cytoplasmic signalling portions of the CD3 and zeta portions of the TCR complex to determine the signalling requirements for activation and death. We have found that the zeta cytoplasmic portion is necessary and sufficient for apoptosis, whereas zeta and the CD3 epsilon intracytoplasmic portion can signal for activation including NF- $\kappa$ B induction and IL-2 expression. Further extensive mutagenesis has shown that specific amino acid sequences called "immunoreceptor tyrosine-based activation motifs" (ITAMS) that are present in the cytoplasmic regions of the various signalling chains have differential effects in inducing activation and death.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00566-06 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Gene Regulatory Events in Establishing Mature T Cell Tolerance**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: **Michael J. Lenardo** Senior Staff Fellow **LI, NIAID**  
 Others: **Behazine Combadiere** INSERM Fellow **LI, NIAID**

COOPERATING UNITS (if any)

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Molecular Development of the Immune System Unit**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**0.5**

PROFESSIONAL:

**0.5**

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects      •(b) Human tissues    X    •(c) Neither  
     •(a1) Minors  
     •(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

T cell tolerance has been found to occur in immature T cells in the thymus and also extrathymically in mature T cells. Our studies of mature T tolerance has revealed that they may undergo deletion or enter a functionally unresponsive state termed anergy. In anergy, a T-helper cell can be induced to "turn off" IL-2 production if it is stimulated through its T-cell receptor in the absence of costimulation. Previously we showed that regulatory proteins that govern IL-2 gene expression may be poorly activatable in response to antigen in anergic T cells. One important determinant of whether T cells will be normally activated by encountering an antigen/MHC complex is whether it is presented along with a co-stimulatory stimulus. Co-stimulation is the signal provided by the interaction of surface molecules such as CD28 (on the T cell) and B7 or BB-1 (on the antigen-presenting cell). The presence of co-stimulation seems to be required for the normal T cell proliferative response to antigen. To understand the role of co-stimulation in IL-2 production, we have studied the effects of co-stimulation on various elements in the IL-2 promoter. Thus far these studies indicate that the transcriptional function of certain cis elements in the promoter can be augmented by co-stimulatory influences. Further studies are in progress to further define the molecular mechanisms involved. Finally, an important aspect of the success of an immune response to an invading micro-organism appears to be the specialization of the helper T cell response towards cells that either produce IL-2 (T<sub>H</sub>1 cells) or IL-4 (T<sub>H</sub>2 cells). We have studied the regulatory elements in the promoter of the IL-4 gene and detected a transcriptional trans-activator and its binding site (CS-1) that are restricted to and crucial for expression in T<sub>H</sub>2 cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00622-04 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**In Vitro Measurement of Binding of Self and Antigenic Peptides to MHC Molecules**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Marie Jelonek	Medical Staff Fellow	LI, NIAID
	Sergei Khilko	Visiting Associate	LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Molecular Biology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**2.0**

PROFESSIONAL:

**2.0**

OTHER:

**0**

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects     
 ☒ (b) Human tissues    X   
 ☒ (c) Neither  
     ☒ (a1) Minors  
     ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this work has been to understand the details of the interaction of self and foreign antigenic peptides with the MHC class I molecule by detailed kinetic and equilibrium binding methods. These studies permit us to understand the underlying biochemical rules that govern peptide/protein interactions, as well as how MHC molecules bind peptides both in intracellular compartments as well as the cell surface.

In the past year we have further developed quantitative assays and have examined a number of peptide/MHC as well as several peptide antibody interactions. Understanding these processes on a biochemical and biophysical level provides a basis not only for understanding how MHC molecules bind and select self and antigenic peptides, but also offers an opportunity for rational design of peptide analogs for both immunization and for intervention in autoimmune disease.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00623-04 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**In Vitro Folding and Assembly of MHC Molecules**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	A. Daniel Platsin	Visiting Fellow	LI, NIAID
	Kannan Natarajan	National Research Council Fellow	LI, NIAID
	Rosemarie Hunziker	Senior Staff Fellow	LI, NIAID

COOPERATING UNITS (if any)

**LICB, NCI (R. K. Ribaud); LB, NCI (M. Mage and L. Lee)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Molecular Biology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**1.0**

PROFESSIONAL:

**1.0**

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues    X    ☒ (c) Neither  
     ☒ (a1) Minors  
     ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project has been to understand the molecular mechanisms that control the assembly and folding of the MHC class I molecules. The MHC class I molecules serve as the critical cell surface structures that sample intracellular peptide fragments for recognition by antigen-specific, MHC-restricted T lymphocytes. In this project we have sought to understand in an *in vitro* system how the components of this three chain structure consisting of MHC class I molecule, synthetic peptide, and the light chain subunit,  $\beta$ 2-microglobulin proceed from linear peptide chains, to a properly folded and biologically active MHC molecule. By engineering single chain analogs of the naturally occurring MHC molecules in which each of the components is part of the same polypeptide chain, we have produced model systems for the efficient *in vitro* folding of class I molecules. Our results indicate that the MHC class I molecule should be considered as a trimer in which each component chain plays a critical role in the pathway to a proper three-dimensional structure. In addition, evidence for an intermediate structure lacking the peptide component, and serologically and functionally distinguishable from the native trimer, has been acquired. Such studies not only permit us to better understand the MHC class I molecule and approaches for engineering variants of it, but also increase our basic understanding of protein folding and its relationship to protein function in general.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
Z01 AI 00624-04 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

New Chemical Tools for Immunology and Structural Biology Research

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John K. Inman Section Chief LI, NIAID  
Others: Patricia F. Highet Technician LI, NIAID

COOPERATING UNITS (if any)

Food & Drug Admin. (B. Golding, H. Golding); State University, Urtecht, The Netherlands (H. Snippe); Lab. Pathology, NCI, NIH (H. C. Krutzsch, D. D. Roberts)

LAB/BRANCH

Laboratory of Immunology

SECTION

Bioorganic Chemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects      •(b) Human tissues    X    •(c) Neither  
    •(a1) Minors  
    •(a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been terminated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00625-04 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Phosphorylation in Effector Functions of T Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. V. Sitkovsky	Section Head	LI, NIAID
Others:	S. Apasov	Visiting Associate	LI, NIAID
	L. Cheng	Visiting Fellow	LI, NIAID
	S. Huang	Visiting Fellow	LI, NIAID
	P. Smith	Research Associate	LI, NIAID
	P. Chen	Research Associate	LI, NIAID

COOPERATING UNITS (if any)

LI (Dr. D. Margulies)

LAB/BRANCH

Laboratory of Immunology

SECTION

Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

4.5

OTHER:

5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects      •(b) Human tissues    X    •(c) Neither  
     •(a1) Minors  
     •(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The antigen-specific cytotoxicity mediated by cytolytic T lymphocytes (CTL) is an important part of immune responses. It is the ultimate goal of our studies to reveal the cellular and molecular requirements for CTL effector functions and differentiation. Our studies of the regulation of T lymphocyte functions by protein phosphorylation are divided into experiments designed i) to test the ectophosphorylation model and ii) to continue the exploration of the role of intracellular protein kinase A (PKA) and PP2a protein phosphatase in CTL generation and functions.

A) Ectophosphorylation Model of T Cell Development and Effector Functions. According to this model the phosphorylation of extracellular domains of cell surface proteins affects cognate lymphocyte cell-cell interactions. It is assumed that the ligand specificity of cell-surface receptors (cell adhesion proteins, recognition molecules [e.g. TCR on T cells, sIg on B cells]) could be regulated through phosphorylation of their extracellular domains in a manner now accepted as a mechanism for the regulation of enzyme-substrate interactions. Use of a panel of monoclonal antibodies and a  $\gamma$ -<sup>32</sup>P-ATP ectophosphorylation assay allowed us to demonstrate the ectophosphorylation of several important surface antigens including TCR, T200, and HSA. We demonstrated the location of the phosphorylated site in the TCR ectodomain using transfectants with GPI-linked T-cell receptor. These data point to the need to evaluate the ectophosphorylation event as potentially regulating TCR affinity for antigen. Work is in progress to determine the exact location of the ecto-phosphorylation site. We have shown that ectoprotein phosphatase activity could be explained by release of the intracellular PP2a enzyme. Experimental tools (cDNA constructs, antisense mRNA oligos) for suspected ecto-enzymes including casein II protein kinase are being developed to directly investigate the role of these proteins in T cell functions.

B) Studies of Intracellular Protein Kinase A and PP2a Protein Phosphatase. Transgenic mice with thymus-specific expression of PKA inhibiting the RI $\alpha$ m subunit have been developed for use in studies of the role of PKA in T cell differentiation. Studies of the PP2a phosphatase led to the demonstration of PP2a association with the plasma membrane in complex with a small subset of high-affinity Con A-binding proteins.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
 Z01 AI 00626-04 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inhibitors of Protein Kinases (or Phosphatases) and Immunomodulators

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. V. Sitkovsky

Section Head

LI, NIAID

Others: S. Apasov

Visiting Associate

LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects

•(b) Human tissues X

•(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of our studies of T lymphocytes is to identify novel and functionally important proteins (enzymes, receptors) and to find their specific inhibitors, agonists, and antagonists. These studies are expected to lead to the development of new immunomodulating drugs. We recently proposed the extracellular ATP model of thymocyte differentiation and peripheral CTL and Th cell effector functions. This, in turn, suggested the possibility of modulating T cell development using ATP analogs. The focus of this project was to investigate the effects of ligands of purinergic receptors (extracellular ATP and adenosine) and their use for immunomodulation purposes. Different ATP analogs and inhibitors of extracellular protein phosphatases were tested using fetal organ thymus culture (FTOC) *in vitro* to model thymocyte differentiation *in vivo*. Dramatic changes in thymocyte subset composition were observed using ectophosphatase inhibitors and purinergic receptor ligands. Incubation with ectophosphatase inhibitors (microcystin) caused strong blocking of differentiation from DN to DP subsets with no major changes in the proportion of CD8<sup>+</sup> SP and CD4<sup>+</sup> SP thymocytes, suggesting that the phosphorylation of ectodomains may be involved in cell-cell interactions leading to thymocyte differentiation. In the presence of ATP, there was a dramatic block of differentiation from DN to DP thymocytes. Slowly-hydrolyzable ATP analogs had effects similar to ATP<sub>0</sub>. Analysis of these experiments supports the view that the major mechanistic components of effects of ATP on thymocyte differentiation in FTOC is transmembrane signaling through purinergic receptors, while processes of ectophosphorylation partially contribute to overall effect. It is concluded that the precise identification of purinergic receptor subclasses involved in thymocyte differentiation using different ATP analogs is not possible, due to the length of the FTOC; however, the observed differences between the effects of adenosine and ATP analogs suggest a differential role for P1 and P2 classes of receptors. Taken together, these studies point to the ATP analogs as promising drugs for immunomodulation.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00627-04 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulatory Events in Cell Development in the Thymus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	L. Yin	Visiting Fellow	LI, NIAID
	J. Zúñiga-Pflücker	Jane Coffin Childs Fellow	LI, NIAID
	D. Jiang	Grad. Student - George Wash. Univ.	LI, NIAID
	P. Schwartzberg	Leukemia Society Special Fellow	LI, NIAID
	C. Trageser	Technician	LI, NIAID

COOPERATING UNITS (if any)

Metabolism Branch, NCI (L. Staudt, H. Varmus); Laboratory of Neurogen, NINDS (H. Arnheiter)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

3.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects      •(b) Human tissues    X    •(c) Neither  
     •(a1) Minors  
     •(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our goal has been to define the important molecular events that control T cell maturation within the thymus. After migrating to the thymus, hematopoietic precursor cells undergo a complex set of developmental events and emerge as mature T lymphocytes capable of specific antigen recognition. This developmental process appears to result from a series of signalling interactions between T cell precursors and a heterogeneous and as yet ill-defined set of stromal cells. We are studying three areas of thymic development at the molecular level. First, we have established a culture system in which day 14/15 triple negative fetal thymocytes can be stimulated to express early markers of thymocyte development including CD25, ICAM-1, and Ly-6A/E. We have found that IL-1 and TNF are critical mediators of progression through the first stage of thymic development leading to expression of the CD25 surface marker. Blockade of these cytokines prevents further maturation of thymocytes to the double positive stage. Our in vitro culture system has allowed us to identify a precursor cell in the thymus that gives rise to T cells, B cells, and natural killer cells. This has allowed us to determine that commitment to the T lineage occurs at the CD25+ stage. Second, we have discovered that the block to the development of double positive T lymphocytes in mice deficient for the recombinase-activating gene (RAG) can be overcome by irradiation. This appears to involve the p53 molecule. Third, we are creating genetically-engineered mice that are homozygous-deficient (knocked-out) for various genes that are highly expressed in thymocytes. We are currently focusing on three genes: i) Ly-GDI - a GDP-dissociation inhibitor protein that controls the activity of Rho, a member of the Ras family that may be involved in molecular signalling cascades; ii) Rlk - a newly described member of the btk/itk family of tyrosine kinases that have been recently implicated in inherited immunodeficiency states and are involved in signalling from surface antigen receptors; and iii) Ntk - a newly-described tyrosine kinase related to the Csk kinase that regulates various Src family kinases by phosphorylating inhibitory tyrosine residues. Homozygous deficient mice have been constructed for LyGDI and Rlk and work is underway on the Ntk knock-out. Since signalling interactions are required for proper T cell differentiation, disruption of these signalling molecules may inhibit T cell development in ways that clarify the normal physiological roles of these molecules.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00628-04**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Antigen-Induced Apoptosis (Propriocidal Regulation) of Mature T Lymphocytes**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	Stefen Boehme	IRTA Fellow	LI, NIAID
	Jeffrey Critchfield	HHMI Scholar - UC-San Francisco	LI, NIAID
	Galen Fisher	HHMI Scholar - Univ PA	LI, NIAID
	Carol Trageser	Technician	LI, NIAID

COOPERATING UNITS (if any)

**Neuroimmunology Unit, NINDS (M. Racke); Alexion Pharmaceuticals (E. Elliott, J. Mueller, L. Matis); LCI, NIAID (W. Strober); LIR, NIAID (G. Panataleo)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Molecular Development of the Immune System Unit**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**3.0**

PROFESSIONAL:

**2.0**

OTHER:

**1.0**

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues    ☒ (c) Neither  
     ☒ (a1) Minors  
     ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is based on our discovery that stimulation of the antigen receptor of mature T cells following exposure of the cells to mitogenic lymphokines such as IL-2 leads to the induction of programmed cell death or apoptosis (propriocidal regulation). We are interested in the mechanism of propriocidal regulation. We are also studying whether this mechanism can explain certain phenomenon of immunological "suppression" that have been previously studied and how this mechanism plays a role in various immunomodulatory strategies being attempted for human disease. We have studied the parameters that control apoptosis during high dose suppression. We found that suppression correlates with IL-2 receptor expression and increased proliferation and was a function of the efficiency of antigen presentation. We have also demonstrated that T cell blasts derived from mice containing a germline deficiency of the p53 tumor suppressor gene are susceptible to TCR-induced apoptosis to the same degree as wild type derived T cells. By contrast, p53-/- T lymphocyte blasts are protected from death caused by the topoisomerase II inhibitors etoposide and teniposide. We also analyzed the expression of Bax and p21 which are induced by p53 in many cell-types and play a role in apoptosis. We found that bax and p21 mRNAs are upregulated in a p53-dependent manner in T cell blasts following stimulation with anti-CD3ε mAb or treatment with the topoisomerase inhibitors. This indicates that the p53-pathway is upregulated when proliferating T cells are stimulated through the T cell receptor, but T cell apoptosis can occur via a p53 independent pathway.

Currently, we are exploring the role of propriocidal apoptosis in tolerance induced by antigen given by different routes including intravenous or intraperitoneal injection or oral administration. Oral tolerance has been proposed as a means to combat various autoimmune diseases by the stimulation of specialized suppressor cells. We are studying whether oral tolerance effects immune responsiveness by causing T cell apoptosis by the propriocidal mechanism. Finally, in AIDS we have been studying the various types of T cell death that occur. We are testing the hypothesis that the loss of selected populations of CD8 T cells that are vital for killing infected CD4 cells may occur by the propriocidal mechanism. Such an event could debilitate the immune response against HIV and lead to progression of disease in AIDS



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
**Z01 AI 00630-04 LI**

PERIOD COVERED

**October 1, 1994 to September 30, 1995**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Role of Cytokines in the Pathogenesis and Treatment of Autoimmune Disease**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. M. Shevach	Section Head	LI, NIAID
Others:	E. Payer	Visiting Fellow	LI, NIAID
	B. Segal	Staff Fellow	LI, NIAID
	F. Topfer	Visiting Fellow	LI, NIAID

COOPERATING UNITS (if any)

**Div Neuropathology, Albert Einstein College of Medicine (C. Raine); LPD, NIAID, NIH (A. Cheever)**

LAB/BRANCH

**Laboratory of Immunology**

SECTION

**Cellular Immunology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

TOTAL STAFF YEARS:

**4.5**

PROFESSIONAL:

**3.5**

OTHER:

**1.0**

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects      •(b) Human tissues    ☒    •(c) Neither  
     •(a1) Minors  
     •(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During T cell development, autoreactive T cells are eliminated in the thymus through a mechanism known as clonal deletion. As a result, mature cells exported to the peripheral lymphoid organs do not react to self-antigens and autoimmune diseases rarely develop. T cells which can recognize self-antigens not expressed intrathymically are not deleted and represent a potential danger. Autoimmunity may be secondary to a failure of intra-thymic deletion of autoreactive T cells, to a failure to induce peripheral tolerance, or to a breaking of established immunological tolerance. Our studies have focused on understanding the pathogenesis of the autoimmune state, the role of cytokines in mediating autoimmune tissue damage, and the treatment of established autoimmune diseases by modulation of the cytokine phenotype of the disease inducing T cells: 1) The mechanisms responsible for inducing tolerance to certain antigens are not completely functional during the first week of age since thymectomy of three day old mice (3dTx) is followed by the development of organ-specific autoimmune disease. The majority of cells in the lymphoid tissues of 3dTx mice appear to have undergone polyclonal T cell activation and demonstrated enhanced responses in the syngeneic mixed leukocyte reaction (SMLR). A direct role for the SMLR reactive T cells in the pathogenesis of disease post-3dTx was demonstrated in studies in which intrathymic tolerization to complexes of self-peptides and MHC class II antigens expressed on adult antigen-presenting cells prevented organ-specific disease. 2) Inflammatory immune responses or Delayed Type Hypersensitivity (DTH) reactions are mediated by CD4+ Th1 T cells which produce interferon- $\gamma$  (IFN- $\gamma$ ), but little IL-4, while CD4+ Th2 populations which produce large amounts of IL-4 and IL-5 mediate immune responses characterized by high levels of non-complement binding IgG, IgE, and eosinophil mediated cytotoxicity, but no organ specific tissue destruction or inflammation. Our recent understanding of the regulation of cytokine production in vitro and in vivo has allowed us to develop protocols for the antigen-specific induction of Th2 populations which may then be used in the therapy of diseases mediated by harmful DTH reactions such as experimental allergic encephalomyelitis (EAE), autoimmune diabetes, uveitis, graft rejection, and contact sensitivity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER  
Z01 AI 00717-01 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Molecular Mechanisms of Autoimmune Disease in Man and Experimental Animal Models**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael J. Lenardo	Senior Investigator	LI, NIAID
Others:	Hugh McFarland	CRADA Fellow	LI, NIAID
	Clara Pelfrey	CRADA Fellow	LI, NIAID
	Galen Fisher	HHMI Scholar- Univ. Of Penn.	LI, NIAID
	Eileen Farnon	Pre-IRTA - Haverford College	LI, NIAID
	Kelly Sung Joe	Pre-IRTA - Univ. Md.	LI, NIAID
	Michele Johnson	Pre-IRTA - Univ. Orego	LI, NIAID

COOPERATING UNITS (if any)

Neuroimmunology Branch, NINDS (H. McFarland); Alexion Pharmaceuticals (L. Matis); LCI, NIAID (W. Strober, S. Straus); NCHGR (J. Puck, F. Rosenberg); Becton-Dickinson (P. Thompson); Exptl Path. Sec. (R. Asofsky)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.75

PROFESSIONAL:

2.25

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects      •(b) Human tissues    X    •(c) Neither  
     •(a1) Minors  
     •(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Based on our work on the mechanism of T lymphocyte or programmed cell death or apoptosis, we have studied autoimmune diseases in two areas: 1) pathogenesis and 2) treatment. We have determined the molecular pathogenesis of one form of autoimmunity by studying a group of children that had been followed by Drs. Stephen Straus and Warren Strober in the LCI, NIAID. These children exhibit an autoimmune/lymphoproliferative syndrome (ALPS) consisting of massive nonmalignant lymphadenopathy, autoimmune phenomena and expanded populations of CD3+, CD4-, CD8- lymphocytes together with antibody-mediated autoimmune disorders. We found that several ALPS children have mutations in the apoptosis-inducing molecule Fas that cause defective Fas-mediated T lymphocyte apoptosis. Transfection studies directly demonstrated that mutant Fas proteins were not only unable to deliver a death signal, but also had a dominant negative phenotype when co-expressed with normal Fas. Family studies showed that the mutations were inherited and thus defined the molecular basis of a genetic autoimmune disorder.

With regard to treatment of autoimmune diseases, we are testing tolerance due to T cell apoptosis as a new modality of immune therapy. A CRADA has been established to test whether T cell apoptosis can be predictably induced by antigen in various animal models of autoimmune disease and graft rejection. The ultimate purpose of these investigations is to advance apoptosis therapy for T cell-mediated diseases to a clinical trial. A primary goal of the CRADA at present is to establish whether the administration of myelin antigens as an antigen-specific therapy can alleviate multiple sclerosis (MS). In preparation for a clinical trial, we have accomplished several things: 1) building and testing recombinant versions of myelin antigens, 2) establishing the relevant animal models to test efficacy and dosing, 3) refining means to test for the elimination of myelin-reactive T cells in peripheral blood. In addition to MS, we have begun work on a transgenic mouse model for myasthenia gravis.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00718-01 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Pathways Involved in the Programmed Death of Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	Stefen Boehme	IRTA Fellow	LI, NIAID
	Lixin Zheng	Visiting Fellow	LI, NIAID
	Galen Fisher	HHMI Scholar - Univ of Penn.	LI, NIAID
	Thomas Yoo	Summer Student - Pomona Coll.	LI, NIAID
	Linda Chen	Summer Student - MIT	LI, NIAID
	Felicita Hornung	Grad. Student - Univ. Freiburg	LI, NIAID

COOPERATING UNITS (if any)

Immunex Research and Development Corp. (Dr. D. Lynch, Dr. Jacques Peschon)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

2.5

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the molecular pathways that govern the programmed death of T lineage cells both during development and in the mature peripheral immune system. Four areas have been addressed: 1) The role of co-stimulatory and co-receptor interactions in modulating a T cell receptor-induced death signal, 2) The involvement of Fas and TNF in mature T lymphocyte death, 3) The involvement of Fas in thymocyte deletion, and 4) Identification of downstream mediators of Fas and TNF-induced apoptosis. We compared the signaling requirements for activation to those required for T cell receptor (TCR)-driven programmed cell death (PCD). Both processes require TCR engagement and ligation of the CD4 co-receptor in the case of a T cell clone that recognizes antigen in the context of an MHC class II molecule. Stimulation of CD28 does not positively or negatively influence TCR-induced PCD, although it was required for IL-2 production. These results provide evidence that in mature T cells there exists a difference in the requirement for CD28 to achieve activation and IL-2 production compared to PCD. We have found that apoptosis in mature T cells can result from interactions between Fas (Apo-1/CD95) and Fas ligand. Defective peripheral T cell deletion due to mutations in the genes for Fas (Apo-1/CD95) and Fas ligand leads to autoimmune diseases resembling human systemic lupus erythematosus in *lpr* and *gld* mice, respectively. We have also demonstrated the occurrence of T cell receptor-induced apoptosis that is Fas-independent, functional in *lpr* or *gld* mice, and acting through tumor necrosis factor. Blockade of both tumor necrosis factor and Fas ligand, but neither alone, abrogates all T cell death. Analyses of mice with homozygous null mutations in the *p55* or *p75* tumor necrosis factor receptor genes show that the *p75* receptor is sufficient for T cell apoptosis. These findings suggest a novel role for tumor necrosis factor and the *p75* receptor in autoregulatory mature T cell apoptosis. In thymocytes, we found synergy between T cell receptor and Fas signalling for apoptosis. This suggests the possibility that Fas plays a role in tolerance induced by the deletion of autoreactive T lineage cells during development. Finally, using yeast-based interaction trap and homology screening methodologies we are studying downstream signalling molecules that play a role in Fas and TNF-mediated T cell apoptosis. We have identified two novel TNF receptor-associated factors or "TRAFFS" and we are studying their role in the death of thymocytes and mature T cells.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AI 00719-01 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

*In Vivo* Analysis of Intracellular Signals Controlling Lymphocyte

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Hua Gu	Section Head	LI, NIAID
Others:	J. R. Hayman	IRTA Fellow	LI, NIAID
	M. Naramura	Visiting Fellow	LI, NIAID
	D. E. Pritchard	Research Technician	LI, NIAID
	J. Y. Lee	Research Technician	LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Development Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.6

PROFESSIONAL:

2.1

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects      •(b) Human tissues    X    •(c) Neither  
     •(a1) Minors  
     •(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Intracellular signalling plays a crucial role in lymphocyte development and function. A panel of molecules involved in signalling has been described and chemical interactions between these molecules documented. However, it remains largely elusive as to whether and how the activation of an individual signalling molecule contributes to various in vivo physiological phenomena such as control of antigen receptor gene rearrangement, repertoire selection, tolerance induction, peripheral lymphocyte activation and maintenance of immune memory. Since these questions can only be properly addressed in an in vivo system, this project focuses on developing animal models by gene targeting and transgenic approaches, and using cellular, molecular and biochemical methods to analyse the impact of various altered signalling molecules on the development, function and pathology of lymphocytes in vivo.

Using classic transgenic and embryonic stem cell technologies, mouse strains transgenic for the CSK tyrosine kinase or a dominant negative form of the Syk tyrosine kinase have been established. To minimize the impact of the transgene expression on the development of animals in early ontogeny, an inducible system was employed to drive the transgene expression. These model systems may allow us to study in detail intracellular signalling mechanisms involved in lymphocyte development and function in a more physiological environment.



**LABORATORY OF IMMUNOPATHOLOGY**  
**1995 Annual Report**  
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**Summary Statement**  
**LABORATORY OF IMMUNOPATHOLOGY**  
**National Institute of Allergy and Infectious Diseases**  
**October 1, 1994 to September 30, 1995**

The interests of this Laboratory are centered on aspects of viral pathogenesis with particular emphasis on the interactions of murine leukemia viruses (MuLV) with the immune system. Retroviruses are studied for their mechanisms of infections of specific cell types, aspects of infection that induce immunodeficiency, and their contributions to lymphoma development. Additional studies are directed at understanding the pathways of hematopoietic differentiation with an emphasis on B cell differentiation. The major accomplishments of the Laboratory in pursuit of these studies are as follows:

Retrovirus-induced immunodeficiency: mouse AIDS (MAIDS) is a syndrome induced by a mixture of murine leukemia viruses that includes an etiologic replication defective virus (BM5def) and nonpathogenic helper viruses (Giese, Holmes, Hartley, Morse). The syndrome is characterized by steadily increasing lymphoproliferation and severe immunodeficiency. Two fundamental aspects of the disease were demonstrated in recent studies of mice carrying gene knockouts that render mice either B cell deficient or incapable of producing MHC class II molecules. These studies showed that B cells are the primary targets for infection by BM5def but that in the absence of B cells, T cells and macrophages are not readily infected (Kim, Tang, Morse). Studies of the class II knockout mice revealed an absolute requirement for expression of these proteins (Giese, Morse).

The requirement for BM5def in disease was investigated by introducing a neomycin resistance gene to the virus genome and generating helper free stocks. Analyses of infected mice showed that MAIDS was induced at low frequencies with long latencies in strains highly susceptible to disease induction by mixed stocks of helper and defective virus. The disease that developed in these mice was often atypical. Transcripts of BM5def were detectable in mice without disease infected helper free. Expression of BM5def thus appears to be necessary but not sufficient for induction of MAIDS (Chattopadhyay, Fredrickson, Morse, Hartley).

A model of AIDS in humans that has attracted much attention suggests that disease progression is a consequence of shifting cytokine patterns from one with a Th1 predominance to one a Th2-dominant profile; evidence suggestive of a similar shift in cytokine profiles was also described for MAIDS. We have examined this model in MAIDS by testing strains of mice that are MAIDS-sensitive but are incapable of producing the Th2 cytokines IL-4, IL-10 or IL-6 as a result of gene knockouts. These studies showed that mice deficient in each of these cytokines or deficient in both IL-4 and IL-10 were no different than wild type mice for development of MAIDS (Morawetz, Giese, Hartley, Morse).

Additional studies of cytokines in MAIDS demonstrated that IFN- $\gamma$  was expressed early after infection and that levels increased with time. Expression of this Th1



cytokine was shown to be important for development of lymphoproliferation as progression of splenomegaly and lymphadenopathy was significantly retarded in IFN-g knockout mice (Giese, Morse). Further studies suggested that enhanced expression of IL-12 is the basis for increased IFN-g expression in infected mice (Giese, Morse). Finally, it was shown that treatment of infected mice with high doses of IL-12 suppressed induction of both lymphoproliferation and immunodeficiency and that this effect was dependent on high level expression of IFN-g (Giese, Morse).

Studies have indicated that inbred strains differ markedly in their susceptibility to MAIDS and that genes of the MHC are major determinants of resistance. Both class I and class II genes contribute to resistance, but the mechanisms for their effects remain unclear. Studies of b2M knockout mice deficient in CD8<sup>+</sup> T cells indicated that this T cell subset is not the major mediator of resistance to MAIDS (Tang, Gabriele, Morse). We also found that immunization of mice with vaccinia viruses recombinant for the BM5def gag gene did not elicit protective immunity (Kulkarni, Dorner, Morse, Chattopadhyay).

Genes outside the MHC can have an overriding influence on disease and crosses between strains that negate the influence of the MHC have been developed that should permit chromosomal localization of the genes and, eventually, their identification (Hartley, Fredrickson, Chattopadhyay, Morse). Studies of mice bearing the *xid* mutation of the *btK* gene revealed that these mice develop MAIDS with a markedly prolonged time course. The basis for this effect appears to lie in abnormalities of conventional B cells from the mutant mice as rapid onset disease was restored by transfer of normal spleen cells (Tang, Kim, Morse).

Two molecular approaches were employed to determine the mechanisms responsible for the activities of the Pr60<sup>gag</sup> of BM5def. First, we attempted to make mice transgenic for the gag gene of BM5def. It appears, however, that the gene may be an embryonic lethal (Chattopadhyay, Morse). Second, we used the yeast two hybrid system to detect protein that might interact with gag. A series of cDNAs were isolated and two have been partially characterized. The most interesting was found to encode a kinesin motor protein, KIF-4 (Kim, Tang, Chattopadhyay, Morse). This protein-protein interaction may be important in movement of gag to the cell membrane prior to particle formation.

In MAIDS-susceptible mice, it was found that clonal populations of B cell and T cells appear regularly within 12 weeks of infection and are transferable to scid mice. All the transfers exhibited clonal integrations of BM5def and most often of ecotropic virus (Tang, Fredrickson, Morse, Hartley). Studies of T cell lymphomas in these mice showed that about 10% of the tumors had virus-associated rearrangements of the Evi-5/Gfi-1 region on chromosome 5 (Liao, Tang, Fredrickson, Hartley, Morse).

**Lymphomas:** A series of mouse strains congenic for ecotropic virus loci of AKR and C58 origin develop high incidences of lymphomas between 12 and 20 months of age. Most have been found to be of B cell origin and include a novel class of splenic marginal zone lymphomas (MZL) described only once previously in the mouse (Hartley, Fredrickson,





Chattopadhyay). Biosy analyses of spleens followed by autopsy at later times showed that some of the MZL progress to more aggressive tumors with centroblastic lymphomas predominating (Fredrickson, Morse, Chattopadhyay, Hartley).

Essentially all the lymphomas have acquired novel ecotropic proviral integrations sites which may serve as markers for genes that contribute to lymphoma induction or progression through insertional mutagenesis. These sites are being cloned to identify affected genes (Liao, Du, Chattopadhyay, Morse, Hartley). As part of these studies, it was found that some of the congenic strains display unexpectedly high frequencies of ecotropic virus integrations resulting in germ cell and somatic mosaicism. Further studies of this phenomenon are in progress (Hartley Chattopadhyay, Fredrickson, Morse).

A gene affected by insertional mutagenesis in AKXD and BXH2 lymphomas has been identified and designated *Evi-5* (Liao). The sequence of a cDNAs has been generated and antibodies to the protein indicate a nuclear localization (Qi, Liao). The mechanism by which altered expression of this gene influences lymphoma development are being actively pursued.

Hematopoietic differentiation: The LIP-6 monoclonal antibody has been proven to be very useful in clarifying patterns of early hematopoietic differentiation and the terminal stages of B cell development. In the bone marrow, LIP-6<sup>+</sup> cells can be subdivided into those that are positive or negative for expression of L-selectin. The L-selectin<sup>+</sup> cells were found to contain a B lineage committed progenitor with a limited self-renewal capacity, a cell type not previously defined. (Holmes).

The mAb to LIP-6 has also been useful in defining late B cells and plasma cells in combination with CD43, L-selectin and B220. LIP-6 was expressed by B cells, immunoblasts and early plasma cells, but not by late plasma cells. Similar patterns were observed for differentiating B cells driven by LPS in vitro or by MAIDS infection in vivo (Holmes).

Virus-cell interactions: Detailed studies of a Moloney MuLV-related retrovirus revealed the appearance of nearly mutually exclusive host range sub-type variants. The responsible determinant was mapped to the env region and sequence analyses revealed a single amino acid difference to be crucial. These findings pinpoint a unique region of the env as important to receptor binding and specificity determination. These findings may have implications for pathogenesis as similar variants appear naturally during the aging process of lymphoma-prone AKR mice (Torrey, Chattopadhyay).

Studies of murine herpesviruses: MHV-68 is a murine gammaherpesvirus that targets B cells and that can apparently contribute to B cell lymphoproliferative disorders including lymphoma long after infection. We are studying this virus to determine if it is a suitable model for EBV infection in humans. The virus is lethal in nude mice, is abrogated in B cell-less mice, and is contained poorly in mice selectively deficient in CD4 or CD8 T cells (Kulkarni, Hartley, Morse).

Studies of mouse cytomegalovirus showed that infection induced striking B cell



activation that was independent of CD4 T cells. The possible contributions of cytokines including IL-6 was suggested by RT-PCR analyses of cytokine transcripts (Karupiah, Hartley, Morse).

Administrative changes: Dr. Andrew Lewis retired from the Laboratory after having been part of the program as Head of the Viral Oncology Section since its inception. Over the years in the PHS, he made many important contributions to our understanding of papovaviruses and their pathogenicity. Dr. Yvonne Eyler, a fellow in Dr. Lewis' section, left to join the U. S. Patent Office. Three new scientists combined to reform the Section; Dr. Xioabei Liao, Dr. Chen-Feng Qi and Dr. Yubin Du. Dr. Wankee Kim completed a Visiting Fellowship in the Virology and Cellular Immunology Section and has taken a position in Korea. Dr. Gunasengaran Karupiah also completed a Visiting Fellowship to return to Australia. The Laboratory was fortunate to bring on Mrs. Gerri Carter as our new Lab Chief's secretary

Special recognition: A day long symposium sponsored by NIAID and NCI was held September 13, 1994 to honor the scientific career of Dr. Janet Hartley, Head, Viral Pathogenesis Section.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

ZO1 AI 00138-18 LIP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Viruses and Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Herbert C. Morse III, M.D., Chief, LIP, NIAID

Others: Janet Hartley, LIP

Arun Kulkarni, LIP

Gunasegaran Karupiah, LIP

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Immunopathology

SECTION

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects    ☐ (b) Human tissues    ☒ © Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project is directed at understanding the mechanisms by which the immune system responds to viral infections. Responses by this system can be divided into components that occur within hours to several days after infection and do not involve antigen-specific responses and later components that involve highly specific responses by T cells and B cells. In many instances, the nonspecific and specific components of the response act synergistically to control infection, but in some cases viruses elicit responses that allow them to evade protective immunity and to persist in the host. We examined the response of mice to infection with mouse cytomegalovirus (MCMV). These studies showed that infection stimulated massive polyclonal B cell activation within 4 days of infection. The response peaked at day 10 and was over by day 14. Activation was associated with increased IgG levels probably secondary to high level expression of interferon gamma. This subversion of the immune system may permit early spread of the virus to the salivary gland where it can remain protected from immune elimination by the late occurring response.

We have also initiated studies of another murine herpes virus, MHV-68 a member of the gammaherpesvirus family. This virus targets lung epithelial cells and B cells for infection, develops latency in B cells and appears to contribute to late onset B cell lymphoproliferative disorders. This virus may serve as a small animal model for acute and chronic infections of humans with EBV.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

ZO1 AI 00284-14

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Characterization of Pathogenic Murine Leukemia Viruses**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Janet W. Hartley, Head, Viral Oncology Section, LIP, NIAID

Others: Herbert C. Morse, LIP  
 Torgny Fredrickson, NCI  
 Sisir Chattopadhyay, LIP  
 Yao Tang, LIP  
 Ted Torrey, LIP

COOPERATING UNITS (if any)

National Institute of Health in Japan, NCI-FCRDC, FDA, Laboratory of Neuroscience, NIDDK

LAB/BRANCH

**Laboratory of Immunopathology**

SECTION

**Viral Oncology Section**

INSTITUTE AND LOCATION

**NIAID, NIH, Bethesda, MD 20892**

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2.0

PROFESSIONAL:

1.0

OTHER:

1.0

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☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project involves the biological and molecular characterization of murine leukemia viruses (MuLV) with the aim of understanding host and viral factors involved in the pathogenesis of neoplastic and non-neoplastic sequelae of infection. A continuing major effort involves studies of the complex of viruses responsible for induction of mouse AIDS (MAIDS), a condition characterized by progressive splenomegaly and lymphadenopathy and immune system impairment; polyclonal lymphoid cell proliferation can progress to oligoclonal or clonal expansions of T and B cells. Disease induction is dependent on a defective MuLV (BM5def) whose only product is an altered gag protein encoded by p15 and p12 sequences. The presence of replication competent helper virus enhances disease induction markedly but MAIDS can develop in mice of sensitive strains infected helper-free, using stocks prepared in packaging cell lines. The occurrence of disease in such mice is sporadic, however, and latent periods are variably extended and disease may be atypical-- in C57BL mice immunoblastic lymphoproliferative disease presenting primarily in thymus was found in most mice with disease and DNA from affected tissues of all displayed J<sub>H</sub> rearrangements and integrated BM5def sequences. Induction of MAIDS requires both B and CD4<sup>+</sup> T cells, and mice with the *xid* mutation develop much delayed disease. Reconstitution experiments indicate that this is due to abnormalities in conventional B cells, not to the deficit in CD5<sup>+</sup> cells also found in *xid* mice.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

ZO1 AI 00286-14 LIP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Janet W. Hartley, Head, Viral Oncology Section, LIP, NIAID

Others: Herbert C. Morse, LIP

Torgny Fredrickson, NCI

Yao Tang, LIP

Ted Torrey, LIP

Sisir Chattopadhyay, LIP, Xiaobei Liao, LIP, Renate Morawetz, LIP

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Immunopathology

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NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

1.5

OTHER:

2.0

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☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project covers studies aimed at expanding our understanding of the pathogenesis of murine leukemia virus (MuLV)-related disease, especially the various genetically specified mechanisms involved in determining sensitivity and resistance of inbred mice to infection and disease induction. Two major areas are under study, one involving exogenous infection of adult mice by the replication defective MAIDS MuLV and replication competent MuLVs, and the second studying the hematopoietic tumors developing spontaneously in NFS V-congenic mouse strains which carry on the virus-negative, low lymphoma NFS background germ-line integrations of ecotropic MuLVs derived from the high virus, high thymic incidence AKR and C58 strains. We have described both MHC Class I and Class II effects on sensitivity and resistance to MAIDS, with a major control deriving from H-2 haplotype. We are now studying genes outside the MHC complex that modify disease and may or may not affect helper virus replication. After extensive strain sensitivity testing, we have established matings and tested progeny of several resistant and sensitive pairs and are utilizing microsatellite DNA mapping to score for genetic markers associated with degree of permissiveness to MAIDS induction and to helper virus replication. In studies of V-congenic mice, lineage relationships established for about 300 tumors indicate that the majority are B cell; 40% of tumors are lymphoblastic or small lymphocyte tumors, 83% of which are of B cell lineage. Over 20% of B cell lymphomas appear to be of splenic marginal zone origin. The majority of tumors tested have new clonal ecotropic MuLV integrations compared to control tissue. Of related interest, in some V-congenic families a surprisingly high level of new clonal proviral integrations has been detected.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00465-10 LIP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Retrovirus-Induced Murine Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Herbert C. Morse III, Chief, LIP

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Laboratory of Immunopathology

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TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

4.0

OTHER:

1.5

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☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Retroviruses cause severe immunodeficiency syndromes in various species including the mouse (mouse AIDS, MAIDS). We are studying MAIDS to develop a basic understanding of how retroviruses induce immune dysfunction. MAIDS is caused by a replication defective virus (BM5def) that encodes a variant 60kD gag polyprotein as its only product; replication competent helper viruses with normal gag polyproteins induce only minor changes in immune function. BM5def gag transcripts are expressed at higher levels in B cells and macrophages than in T cells and expression in B cells is associated with abnormal signaling after immunoglobulin crosslinking. However, expression in lymphocytes is not sufficient to induce disease as mice deficient in expression of major histocompatibility complex class II molecules express BM5def gag at high levels but do not develop MAIDS. Infection with BM5def results in enhanced expression of the cytokines IL-4, IL-10, and IFN- $\gamma$ . MAIDS develops normally in mice with gene knockouts for IL-4, IL-10 or both cytokines, indicating that expression of these two cytokines is not required for disease. In contrast, disease develops more slowly in mice with an IFN- $\gamma$  knockout. Studies designed to investigate therapeutic strategies showed that immunization of mice with vaccinia carrying BM5def gag was ineffective. However, administration of IL-12 to infected mice prevented or greatly reduced the extent of virus-induced immune abnormalities. These results indicate that MAIDS results from a complex interplay of BM5def with cells of the immune system. Current studies are focused on determining if BM5def gag binds to particular cellular proteins and what interactions might affect cell signaling pathways that are altered during the course of disease.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00484-09 LIP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms in Hematopoietic Cell Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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3.25

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2.0

OTHER:

1.25

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☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The LIP-6 monoclonal antibody identifies a novel protein expressed by mature B and myeloid lineage cells and by bone marrow pre-B cells. We have investigated whether LIP-6 can be used to isolate cells that are committed to B cell differentiation but lack the expression of CD45R(B220). The phenotypic identification of cells prior to CD45R(B220) expression would enable the study of mechanisms responsible for hematopoietic cell lineage commitment. A population of bone marrow cells has been identified that is LIP-6<sup>+</sup>CD45R(B220)<sup>-</sup> Ly-5(CD45)<sup>+++</sup> that has the capacity to differentiate into B, T and myeloid lineages when inoculated into sublethally irradiated mice. This population was further divided phenotypically into L-selectin<sup>+</sup> and a L-selectin<sup>-</sup> populations, comprising 3-4% and 0.5%, respectively, of total bone marrow. In vivo reconstitution studies with these cells has shown that the L-selectin<sup>+</sup> cells differentiate only into B lineage cells but have a limited capacity for self renewal. In contrast, the L-selectin<sup>-</sup> cells were capable of differentiation within the B, T and myeloid lineages and probably contains pluripotent hematopoietic stem cells. Future experiments will determine the frequency of B cell progenitors within the L-selectin<sup>+</sup> cells as well as their other phenotypic and functional characteristics. LIP-6, in combination with CD43, L-selectin, and CD45R(B220), has also been used to delineate the terminal stages of B cell differentiation. LIP-6 expression is increased during the immunoblast, plasmablast and immature plasma cell stages, but is lost upon differentiation to the mature plasma cell. In addition, L-selectin<sup>-</sup> LIP-6<sup>+</sup> cells found in Peyer's patches and immunized lymph nodes correspond phenotypically to germinal center B cells. Experiments to verify this are in progress. In addition, attempts to isolate and clone the gene coding for the LIP-6 protein will continue. A B cell line, NFS-1.0, that expresses LIP-6 will be used to construct a cDNA expression library to be screened with the LIP-6 antibody.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00544-07

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Basis of Pathogenesis of Murine Leukemia Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sisir K. Chattopadhyay, LIP

Others: H.C. Morse, LIP

Torgny Fredrickson, NCI

J.W. Hartley, LIP

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Yao Tang, LIP

WanKee Kim, LIP

Arun Kulkarni, LIP

Michael Potter, NCI

COOPERATING UNITS (if any)

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Laboratory of Immunopathology

SECTION

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

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☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Murine AIDS (MAIDS) is caused by a replication defective murine leukemia virus (MuLV) called BM5def. BM5def was molecularly cloned and sequenced to reveal that the virus has a functional LTR and with a single open reading frame in gag gene that encodes a variant Pr60<sup>gag</sup>. This protein differs from the gag polyprotein of a nonpathogenic ecotropic virus in the carboxyterminus of MA(p15) and throughout much of a p12. The primary purpose of this project is to determine how this single protein contributes to the development of lymphoproliferation and immunodeficiency.

Numerous approaches were taken to generate mice expressing BM5def-gag from a transgene. Injection with gag sequences driven by SV40 promote IgH Eμ enhancer or MHC Class II Eα promoter yields mice bearing the transgenes, but often with no expression of mRNA and in mice having transcripts, no protein. More recently, injections were made with BM5def genomic DNA, again without success. Other laboratories have also failed to generate transgenics using these and other approaches suggesting that the gag gene product may be an embryonic lethal.

Vaccinia recombinants bearing the BM5 ecotropic and defective virus gag gene were generated that express the encoded proteins at high levels. Mice immunized and boosted with these vectors were unchanged in their sensitivity to MAIDS. Molecular clones of the BM5 defective and ecotropic viruses were used to generate "bait" for the yeast two-hybrid system to fish out proteins that might bind selectively to BM5def-gag. Two proteins appeared repeatedly in the search and both, when expressed as GST fusion proteins, bind to BM5def-gag expressed from the vaccinia recombinants. The genes encoding these proteins are provisionally designated Defective gag-associated protein 1 (Dgap-1) and Dgap-2.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00692-03

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Determinants of Murine Leukemia Virus Pathogenicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

Ted Albert Torrey, LIP

Janet W. Hartley, LIP

Sisir K. Chattopadhyay, LIP

Torgny N. Fredrickson, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

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SECTION

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TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

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☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project aims to identify some of the molecular and cellular determinants involved in murine leukemia virus (MuLV) infectivity and pathogenesis by the establishment of *in vitro* systems for host range sub-type characterization and lymphoid cell transformation. We have demonstrated by site-directed mutagenesis that a single amino acid change in the envelope protein of an ecotropic MuLV can radically alter the infective host range, *i.e.* resulting in the ability to infect a mouse cell line restrictive to the parental MuLV while greatly diminishing infectiousness for mouse cell lines permissive to the parent virus. This alteration in host range sub-type is independent of host cell functions, although the magnitude of restriction of infection can be influenced by host cell properties. The effects of this single amino acid change, and a series of similar alterations in the same envelope protein structural element, on receptor binding and utilization are currently being tested. In addition, in order to develop a model system for the lymphomagenicity of MuLV *in vivo*, we have established a cell culture system which is apparently permissive for MuLV-mediated lymphoid cell transformation. Normal mouse neonate thymocytes can be induced to persist and expand in culture supported by MuLV-infected and expressing thymic stromal cell lines; this capacity is greatly augmented by mink cell focus-inducing (MCF) viruses, MuLV known to accelerate lymphomagenesis *in vivo*. We are exploiting this system to dissect the viral and cellular determinants of susceptibility to retroviral transformation.



Laboratory of Immunoregulation  
1995 Annual Report  
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Summary Report  
Laboratory of Immunoregulation  
October 1, 1994 through September 30, 1995

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The major theme of the Laboratory of Immunoregulation (LIR) is the study of the cellular and molecular mechanisms of the regulation of the human immune response in health and disease. Over the past several years, the LIR has focused the major component of its efforts on the delineation of the immunopathogenic mechanisms of human immunodeficiency virus (HIV) infection and disease while maintaining its commitment to the study of the broader area of human immunoregulatory mechanisms. It is our belief that only by appreciating in a comprehensive manner the complex immunopathogenic mechanisms of HIV disease can we formulate a solid scientific basis for the design of therapeutic and vaccine strategies. We have focused on the host factors involved in the evolution of HIV disease and have developed a model of disease progression whereby the complex pathogenic mechanisms are multifactorial and multiphasic, and these phases overlap throughout the prolonged course of disease. In this regard, we have focused our efforts on the various stages of HIV disease from acute primary infection, to the period of prolonged clinical latency and ultimately advanced disease, and have studied the immunopathogenic mechanisms at the tissue, cellular, cytokine, and molecular levels. In addition, we have designed and execute therapeutic and vaccine protocols.

Virologic and Immunologic Events Associated with Primary HIV Infection

Despite the fact that a large proportion (up to 70%) of individuals experience a non-specific, mononucleosis-like clinical syndrome, hospitalization is required in only a minor percentage of individuals. Because of these logistic constraints, it has been difficult to obtain lymph nodes biopsies during acute infection in humans, and for obvious reasons it is not possible to perform longitudinal studies. Therefore, although the virologic events in peripheral blood (i.e., high levels of viremia and of HIV DNA and RNA synthesis in peripheral blood mononuclear cells [PBMC]) associated with primary HIV infection have been delineated, evidence for the initial localization of HIV in lymph nodes in humans is only indirect. In this regard, the simian immunodeficiency virus (SIV) animal model of acute infection represents an excellent experimental system to address this issue. The execution of sequential biopsies has demonstrated localization of SIV (i.e., numerous individual infected cells) in peripheral lymph nodes as early as day 7 post-inoculation. Trapping of virions in the follicular dendritic cell (FDC) network was detected by week 2 post-inoculation and increased over time, whereas the number of cells expressing SIV dramatically decreased. Trapping of virions coincided with an increase in the levels of antibodies that efficiently bind complement. It is highly likely that similar events occur in humans. These studies indicate that the initial establishment of HIV infection occurs in the lymphoid organs. More importantly, these viral particles trapped in the FDC network during primary infection may be critical for propagation of infection over time and may represent a mechanism of virus escape from the immune response. Cross-sectional analysis of HIV distribution has been performed in lymph nodes obtained from patients with primary infection between 3 and 30 weeks from the onset of symptoms. The kinetics





of HIV distribution in lymph node during primary infection were similar to those observed in the SIV model.

Both vigorous HIV-specific cell-mediated and humoral immune responses are present during primary infection and certainly play a critical role in the downregulation of viremia. However, these immune responses are unable to totally clear HIV. Therefore, it is critical to delineate more precisely the nature of the primary immune response to HIV infection in order to determine the mechanisms responsible for the lack of efficacy of HIV-specific immune responses. To characterize the primary cell-mediated immune response, we have analyzed the T cell receptor (TCR) repertoire during primary HIV infection. In a longitudinal study of HIV-infected individuals following primary HIV infection, major expansions in a restricted set of V $\beta$  families have been observed. The cells expressing the expanded V $\beta$ s are predominantly CD8<sup>+</sup> T lymphocytes, they mediate HIV-specific cytotoxic activity against HIV proteins, and they are involved in the expression of certain cytokines. Furthermore, nucleotide sequences of the recombinant clones of the expanded V $\beta$ s has demonstrated the oligoclonal nature of these expansions. These results indicate that major oligoclonal expansions of CD8<sup>+</sup> T lymphocytes represents an important component of the primary immune response to HIV infection. Furthermore, on the basis of the longitudinal analysis of the changes of the TCR repertoire performed in a large number of HIV-infected individuals with primary infection, three patterns of V $\beta$  expansions were observed, and these patterns of V $\beta$  expansions were associated with different rates of progression of HIV disease. These results indicate that there are qualitative differences in the primary immune response to HIV that may be associated with more effective versus less effective control of HIV disease progression. (Pantaleo, Graziosi, O Cohen, Muro-Cacho, Vaccarezza, Fauci, LIR/NIAID; Fox, Molecular Histolabs, Gaithersburg, MD; Orenstein, George Washington Univ; Baseler, PRI, Frederick, MD; Kotler, St. Luke's-Roosevelt Hospital, New York, NY; Shaw, Saag, Univ of Alabama/Birmingham)

### The Role of Cellular Activation in the Immunopathogenesis of HIV Disease

One of the major characteristics of HIV infection is the intense degree of cellular activation that occurs throughout the course of HIV disease. Cellular activation plays a major role in the pathogenesis of HIV disease since HIV replicates much more efficiently and spreads more readily among activated cells. In addition, persistent immune activation contributes to the immune defects in HIV infection by inducing anergy and/or apoptosis among activated cells. We have investigated the occurrence of apoptosis *in vivo* in lymph node sections obtained from HIV-infected individuals at different stages of disease. In addition, the degree of apoptosis in HIV-infected lymph nodes was compared with that observed in lymph nodes obtained from HIV-negative individuals. Apoptosis has been readily detected in lymph nodes obtained from both HIV-negative and HIV-infected individuals. However, the degree of apoptosis in lymph nodes obtained from HIV-infected individuals was three to four times higher than that observed in the lymph nodes obtained from HIV-negative individuals; furthermore, in HIV-infected lymph nodes all functional compartments of the lymph node (i.e., cortex, paracortex, and sinuses) were extensively involved by this phenomenon. A significant correlation was observed between apoptosis, the degree of activation of the lymphoid tissue, and the chronic immune activation associated with HIV infection. In contrast, apoptosis intensity correlated neither with clinical stages of HIV disease nor with viral burden in lymph node. Finally, apoptosis was not restricted only to CD4<sup>+</sup> T cells; both B cells and



CD8<sup>+</sup> T cells undergo apoptosis. Taken together, these results indicate that the increased intensity of the apoptotic phenomenon in HIV infection is caused by the general stage of immune activation; however, it is independent of the progression of HIV disease and of the levels of viral load.

We have also investigated the effects of cyclosporin A (CsA) on HIV infection *in vitro* and in the SIV animal model of acute infection. CsA inhibits HIV infection *in vitro* at concentrations of drug easily tolerated in clinical practice. CsA cooperates with zidovudine (ZDV) in suppressing HIV infection *in vitro*. In acute SIV infection, CsA administration delays the onset of viremia, prevents the drop in CD4<sup>+</sup> T lymphocytes, does not increase the percentage of CD8<sup>+</sup> T lymphocytes, and potentiates antibody responses. Apoptosis intensity is reduced in lymph nodes of SIV-infected monkeys treated with CsA compared to that observed in lymph nodes of acutely infected untreated monkeys. These studies indicate that inhibition of the state of the heightened immune activation in SIV/HIV infection may have a beneficial effect and may serve as the scientific basis for feasibility studies in HIV infection in humans. (Pantaleo, Muro-Cacho, Vaccarezza, Fauci, LIR/NIAID; Orenstein, George Washington Univ; Fox Molecular Histolabs, Gaithersburg, Md; Baseler, PRI, Frederick, MD; Martin, Tulane Univ; Diiffenbach, Black, Sager, DAIDS/NIAID)

### The Role of Exogenous Activation of the Immune System in the Pathogenesis of HIV Disease

We have evaluated the role of immune activation in HIV infection using the SCID-hu mouse as well as patient studies. In the SCID-hu mouse, infection with HIV, which results in thymocyte depletion and destruction of the thymic microenvironment, could be completely blocked or significantly inhibited when mice were treated with CsA before and during the course of infection. This inhibition of infection was associated with a decrease in the expression of the CD25 activation marker on the mature CD3<sup>+</sup> thymocytes, suggesting that the mechanism of viral inhibition might be related to inhibition of activation. Further studies are ongoing to determine more definitively the mechanism of inhibition by CsA.

In both HIV-infected and uninfected individuals we have studied the effects of a discrete immune stimulus, namely a tetanus toxoid booster injection, on acute infection of uninfected cells, virus expression, and immunologic response. In the infected individuals we observed a variable, but generally blunted, tetanus specific immune response following the booster injection, with minimal proliferation to tetanus and small to normal tetanus immunoglobulin (Ig) production. However, the immune systems of these patients became activated, as indicated by increases in the expression of HLA-DR on CD4<sup>+</sup> T cells. In most cases the efficiency of viral isolation *in vitro* was increased after immunization, indicating that the *in vivo* activation renders the cells more likely to produce HIV *in vitro*. Likewise, plasma viremia increased in all patients, peaking usually at about 3 weeks after booster and returning to baseline by 6 weeks. The greatest changes in viremia and virus isolation occurred in those patients who were capable of making the greatest immune response to tetanus, and thus appeared to correlate with the degree of activation of the immune system by the tetanus booster. Uninfected individuals responded to tetanus booster. Uninfected individuals responded to tetanus booster immunization with a vigorous cellular (proliferation) and humoral (Ig production) immune response as expected. In these individuals, the ability to acutely infect their PBMC *in vitro* increased dramatically after immunization and often did not require the addition of exogenous stimuli to the cultures, indicating that the *in vivo* activated cells were more susceptible to infection and probably constitutively producing interleukin (IL)-2. Thus, activation



induced by a booster immunization results in increased *in vivo* production of virus in HIV-infected individuals and increased susceptibility to infection in seronegative individuals. These findings have important immunopathogenic and public health implications. Further work is ongoing to determine the nature of the abnormal immune response. The effects of anti-retroviral drugs in these individuals, and the role of apoptosis in this process. (Stanley, Ostrowski, Fauci, LIR/NIAID)

### The Role of Mycobacteria Tuberculosis in the Pathogenesis of HIV Disease

Mycobacterial tuberculosis (MTB) disease continues to be the most important life-threatening bacterial disease in the world today with 8 million new cases and 3 million deaths reported each year to the World Health Organization. The prevalence of MTB disease has recently increased in part due to HIV infection. In fact, epidemiological data have demonstrated that HIV-infected individuals are more susceptible to MTB infection and disease. Moreover, it has been shown that MTB disease causes an acceleration in the progression of HIV disease. The purpose of this study was to delineate how MTB modulates HIV infection *in vivo* and in *in vitro* models. We measured plasma viral load, a direct reflection of lymphoid viral replication, in HIV-infected individuals before, during, and after MTB disease. MTB increased the plasma viral load of HIV-infected individuals during the acute phase of MTB disease compared to before the onset of the disease and after treatment. To evaluate the mechanisms involved in the MTB-induced augmentation of HIV replication, we studied the virologic and immunologic responses induced by MTB and the constituent antigen PPD in an *in vitro* system using primary PBMC and lymph node cells isolated from HIV-infected individuals. The data demonstrated that MTB induced HIV replication in CD8 depleted lymphocytes of HIV-infected individuals who had a history of PPD positivity, in the absence of exogenous stimulation. The increase in HIV production mediated by MTB or PPD correlated with the level of cellular activation as demonstrated by an expansion of CD4<sup>+</sup>, CD25<sup>+</sup> cells and by an increase in cellular proliferation. In addition, we have demonstrated that MTB and PPD increased viral replication in an acute infection model where PBMC from healthy donors who were either skin test positive or negative for PPD were infected with HIV-1 primary isolates and this effect was also correlated with the level of cellular activation and proliferation. In conclusion, MTB increases viral replication *in vivo* and in an *in vitro* model. This MTB-mediated viral production likely occurs through the activation and infection of responding T cells. We believe that these findings may be important to further delineate the immunopathogenic mechanisms of HIV disease and to develop therapeutic strategies based on these mechanisms. (Goletti, Weissman, Fauci, LIR/NIAID; Klein, Munsiff, Montefiori Medical Center; Graham, Hopkins; Ortona, Cauda, Catholic University of Rome)

### Cytokine Expression in HIV Disease

We have analyzed the constitutive expression of cytokines in peripheral blood and lymph nodes obtained from the same patients. Expression of a panel of cytokines, including IL-2, IL-4, IL-10, and interferon (IFN)- $\gamma$  in unfractionated or sorted T-cell populations isolated from peripheral blood and lymph nodes of HIV-infected individuals at different stages of HIV infection was determined by a semi-quantitative polymerase chain reaction (PCR) assay. In addition, cytokine expression was determined in purified CD4<sup>+</sup> T-cell populations after stimulation *in vitro*.



Constitutive expression of cytokines in peripheral blood and lymph node was significantly higher in HIV-infected individuals compared to HIV-negative individuals. These findings are consistent with the general state of immune activation associated with HIV infection. Both cross-sectional and longitudinal analyses of constitutive cytokine expression in peripheral blood of HIV-infected individuals at different stages of disease indicated that there were no obvious changes in the pattern of cytokine expression during progression of HIV disease. Constitutive cytokine expression was barely detected or absent in CD4<sup>+</sup> T cells. CD8<sup>+</sup> T cells were predominantly involved in the constitutive expression of IFN- $\gamma$  and, to a lesser extent, of IL-10. No change in the pattern of cytokine expression was observed at the different stages of disease following stimulation of purified CD4<sup>+</sup> T cells *in vitro*. These results do not support the hypothesis that a switch from a T<sub>H</sub>1 to a T<sub>H</sub>2 cytokine pattern is associated with progression of HIV disease.

High levels of pro-inflammatory cytokines including IFN- $\gamma$ , tumor necrosis factor (TNF)- $\alpha$ , IL-10 and, to a lesser extent, IL-6 were associated with primary HIV infection. Expression of IFN- $\gamma$  coincided with the expansion of CD8<sup>+</sup> V $\beta$  cell subsets, whereas later activation of B cells and macrophages were responsible for the expression of the other cytokines. (Graziosi, Pantaleo, O Cohen, Fauci, LIR/NIAID; Sekaly, Fortin, IRCM, Montreal)

### The Role of Cytokines in the Regulation of HIV Expression

For many years the LIR has been investigating the role of cytokines in the regulation of HIV expression. Identification of HIV-modulating cytokines and the delineation of the mechanisms by which they act was accomplished primarily by analyzing the effect of exogenously added cytokines on HIV expression in chronically infected cell lines of both monocytic and lymphocytic origin. We continue to perform such work; however, this year we have focused on demonstrating these concepts on a more physiologic basis. The ability of various physiologic stimuli to induce the production of endogenous cytokines which then act in an autocrine/paracrine manner to modulate viral production from infected cells were demonstrated in several culture systems such as IL-2-stimulated PBMC acutely infected with HIV and endotoxin-stimulated monocytic cell lines chronically infected with HIV. Suppression of HIV expression in these systems was effectively achieved by treatment of infected cells with a variety of physiologic inhibitors of endogenous cytokine production, such as anti-proinflammatory cytokines, as well as by natural antagonists of cytokine activity, e.g., soluble cytokine receptors and IL-1 receptor antagonist. CD30, a member of the TNF receptor family, is expressed constitutively on the surface of the human T-cell line ACH-2 that is chronically infected with HIV-1. We demonstrate that cross-linking CD30 with an anti-CD30 specific monoclonal antibody, which mimics the described biological activities of the CD30 ligand (CD30L), results in HIV infection. CD30 cross-linking does not alter proliferation of ACH-2 cells and the induction of HIV expression is not mediated by endogenous TNF- $\alpha/\beta$ . Furthermore, cross-linking of CD30 leads to NF- $\kappa$ B activation and enhanced HIV transcription. Thus, CD30-CD30L interactions mediate the induction of HIV expression by a  $\kappa$ B-dependent pathway that is independent of TNF. This mechanism may be important in the activation of HIV expression from latently infected CD4<sup>+</sup> T cells especially in lymphoid organs where cell to cell contact is conducive to receptor-ligand interactions. The focus of our research this year is to analyze the effects of various immunoregulatory cytokines, in particular, IL-2 and IL-12, on HIV replication in PBMC and lymph node (LN) MC of infected individuals. These cytokines are in clinical trials, yet little is known of their effect on virus replication in the context of the immune status





during HIV disease. Expectedly, both IL-2 and IL-12 stimulated HIV replication in purified CD4<sup>+</sup> cells obtained from HIV-infected individuals; however, in unfractionated cell cultures IL-12, but not IL-2, allowed virus isolation. We found that IL-2 was significantly more effective than IL-12 in stimulating CD8-mediated suppression of HIV replication and that this effect overrode the stimulatory effect of IL-2 on HIV production by infected CD4<sup>+</sup> cells. These findings are of particular interest in light of results from the IL-2-based clinical trial conducted by other sections of the laboratory in which it was found that the increases in plasma viremia immediately following administration of IL-2 were consistently reduced to baseline levels despite a sustained expansion of the primary targets of HIV infection, CD4<sup>+</sup> lymphocytes. These studies demonstrate that physiologic stimulation of infected cells can lead to the production of cytokines or soluble factors capable of either enhancing or inhibiting HIV production from infected cells. Such findings further our understanding of the complex role cytokines play in modulating the levels of HIV replication and spread whether by acting directly on infected cells and/or indirectly by influencing anti-viral activities of cells which are not targets of HIV infection. (Kinter, Goletti, Bende, Biswas, Fauci, LIR/NIAID)

### The Role of Dendritic Cells in the Pathogenesis of HIV Disease

We have been studying the role of bone marrow derived dendritic cells (DC) in the pathogenesis of HIV disease. DC are likely involved in both the initiation and propagation of HIV infection. They are the first immune cells to arrive at sites of inflammation on mucous membranes, the major site of sexual spread of HIV. They function by obtaining antigens, transporting them to lymphoid organs, and initiating T-cell immune responses. We have previously demonstrated that DC can bind HIV for extended periods of time and induce infection in unstimulated autologous CD4<sup>+</sup> T cells. HIV replication continues throughout the course of disease in the paracortical regions of lymphoid organs which are comprised of DC and T cells. It has been demonstrated that HIV production comes from acutely infected T cells likely via antigen driven activation. These data suggest that DC in the lymph node drive the T-cell activation that leads to viral replication. We have developed a model of the cellular interactions of the paracortical regions of lymphoid organs using DC and unstimulated CD4<sup>+</sup> T cells to study the role of cytokines, immune activation, and activities produced by CD8<sup>+</sup> T cells on viral replication and HIV pathogenesis. CD8<sup>+</sup> T cells have been observed to inhibit HIV replication in stimulated PBMC systems. Using DC-T cell co-cultures, we have observed that there are at least 2 sets of CD8<sup>+</sup> T-cell activities. One activity is produced by CD8<sup>+</sup> T cells from HIV-infected and uninfected individuals. This activity can inhibit DC-induced viral replication in CD4<sup>+</sup> T cells from HIV-infected people (endogenous system), but has not activity in an acute infection model where DC from HIV-negative people are pulsed with HIV and added to autologous CD4<sup>+</sup> T cells. The second activity has only been found in CD8<sup>+</sup> T cells from HIV-infected people. It functions in the acute infection model and is radiation sensitive. Further studies to identify and characterize these activities are underway. The DC-T cell co-culture system has also been used to study the role of an activated immune system and HIV infection. African HIV disease is characterized by a greater rate of infection per exposure and a shorter survival of infected people, which is thought to be due to an activated immune phenotype. We used tetanus immunization as a mode of activation and found that during the peak of an immune response DC could induce infection with 25 to 100 times less virus. Thus, using a physiologic model, we have demonstrated a possible mechanism for the enhanced HIV infection observed in people with activated phenotypes. Further work will explore the effect of vaccination with HIV antigens with regard to a possible subsequent enhancement of infection of CD4 positive T cells (Weissman,



Barker, Li, Ananworanich, Fauci, LIR/NIAID; Baseler, PRI, Frederick, MD; Orenstein, George Washington Univ)

### The Role of Follicular Dendritic Cells in the Pathogenesis of HIV Disease

Lymphoid tissues are the major reservoir and site of viral replication of HIV. The destruction of the FDC network has a major impact on lymphoid tissue architecture and function in HIV-infected individuals; yet, the mechanisms involved in this process are not understood. Our studies have demonstrated that FDC are bone marrow derived and share a common precursor with B cells. We have also created an EBV-like cell line. Our preliminary data on this cell line have shown that it can induce HIV expression in latently infected cell lines and bind to complement and antibody coated HIV particles through complement receptors similar to primary FDC. We plan to further identify the FDC precursor cells and to study the role FDC in viral expression and propagation in HIV-infected patients. (Li )

### Effect of Therapy on HIV Viral Load in Lymph Nodes

Lymphoid organs are the major *in vivo* reservoir of HIV-1. The effects of standard antiretroviral therapy on HIV burden (i.e., the frequency of HIV-infected cells) and expression were evaluated by sampling peripheral blood (PB) and lymphoid tissue (LT) of patients before and 8 weeks after either remaining untreated, remaining on ZDV, initiating ZDV, or adding ddl to ZDV. In individuals who did not undergo a change in therapy, patterns of histopathology, viral trapping, viral burden, and viral replication remained remarkably constant between week 0 and week 8. In the group which added ddl to zdv therapy, decreases in plasma viremia coincided with decreases in virus replication in LT. Although decreases in viral expression were seen in this group of patients, the total pool of HIV-infected cells remained essentially unchanged. The decrease in viral expression without change in viral burden implies that a relatively small population of newly infected cells accounts for the majority of active viral replication at any given point during the course of HIV disease.

The effects of an immunomodulatory agent, dinitrochlorobenzene (DNCB), in combination with Chinese herbs were evaluated by sampling PB and LN at baseline and after 6 months of initiating DNBCB and herbs (DNCB treatment group) or herbs alone (control group). A significant decrease in CD4<sup>+</sup> T-cell counts was noted in both groups. Total CD8<sup>+</sup> T-cell counts remained unchanged; however, there was a significant increase in the percentage of CD8<sup>+</sup>CD38<sup>+</sup> cells in both groups. Viral burden and viral replication in PB and LN remained stable in both groups. Analysis of cytokine (interferon- $\gamma$ , TNF- $\alpha$ , IL-2, IL-4, IL-6, IL-10) expression from PBMC and LNMC revealed a TH<sub>0</sub>-like pattern at baseline which did not change at the 6-month time point in either group. These results thus failed to show any promising virologic or immunologic effects of DNBCB therapy in patients with intermediate-to-late stage HIV disease. (O Cohen, Pantaleo, Graziosi, Vaccarezza, Fauci, LIR/NIAID; Orestein, George Washington U; Fox, Molecular Histolabs, Gaithersburg, MD; Pavlakis, Schwartzentruber, FCRDC/NCI; Schnittman, DAIDS, NIAID; Holodniy, Palo Alto VA Medical Ctr; Loveless, U Oregon; Moss, Immunization Products, CA).



## Studies on HIV-Infected Long-Term Non-Progressors (LTNPs).

We have studied a group (n=23) of HIV-infected individuals whose disease has not progressed over periods ranging from 7 to 18 years. These individuals have been termed LTNPs in that they have been known to be HIV-infected and have been followed with serial (every 6 months) CD4<sup>+</sup> T-cell counts for at least 7 years. In addition, they have not received anti-retroviral therapy. To gain greater insight into potential immunopathogenic mechanisms of HIV disease, we studied these patients from a virologic and immunologic standpoint with particular emphasis on their lymphoid tissue. We had previously demonstrated that lymphoid organs represent a major reservoir for HIV viral burden and are the primary sites for virus replication. In addition, we have previously demonstrated that lymphoid tissue (immune competent cells and lymph node microenvironment) is progressively destroyed as HIV disease progresses from early to advanced stages. In early stage disease, lymph nodes undergo hyperplasia and extracellular virions are trapped on the FDC of the activated and hyperplastic lymph node germinal centers. This is coincident with the partial clearance of viremia, although viral replication persists throughout all stages of HIV infection. As disease progresses (usually over years), the lymphoid tissue involutes, extracellular virus is no longer efficiently trapped, virus replication in individual cells increases, and plasma viremia increases. Using this model of disease progression as a framework, we studied our group of LTNPs. Levels of viral burden and virus replication were quite low in PB and LN MC of LTNPs compared to progressors. Of interest was the fact that relatively high levels of plasma viremia were noted in the majority of LTNPs, comparable to levels in HIV-infected individuals whose disease had progressed. Quantitative analysis of viral mRNA produced in LNMC revealed significantly lower levels of multiple-spliced HIV RNA as well as single-spliced plus unspliced RNA in LTNP compared to progressors. There was no significant difference between LTNP and progressors, however, in the ration of multiple-spliced to single-spliced plus unspliced RNA levels. Qualitative analysis of multiple-spliced RNA species from LNMC revealed more complete expression in progressors compared to LTNP. Most commonly, LNMC from LTNP did not express one of the *rev/nef* messages. Despite this intriguing finding, it is clear that in the majority of LTNP, HIV mRNA synthesis proceeds in a manner which does allow for expression of all HIV messages due to the redundancy of *rev/nef* messages within HIV. The size of the LNs of LTNPs were generally smaller than those of progressors and the degree of follicular hyperplasia and the total nodal and germinal center areas were significantly less in LTNPs. Of note, and in striking contrast to progressors, the lymph node architecture of the LTNPs was preserved despite several years of infection. Variable degrees of virus trapping was detected; several patients had little if any trapping of extracellular virions. Virus particles were virtually never detected in tissue or cell suspensions by electron microscopy. Only rarely were individual cells detected that were expressing HIV. The lack of virus trapping could in part explain the fact that although only very few cells were actively producing virus in LN, plasma viremia seemed not to be efficiently cleared leading to relatively high levels of plasma viremia (see above). These data together with studies of lymphoid tissue in progressors suggests that disease progression is at least in part related to the deposition of virions on the FDC of LN germinal centers, persistent activation of LN, active virus replication, and progressive destruction of lymphoid tissue. From an immunological standpoint, HIV-specific cytotoxicity against gag proteins was consistently observed in PBMC of LTNPs. Proliferative responses to a variety of stimuli (mitogens, alloantigens, and recall antigens) were preserved in PBMC of LTNPs. Characterization of humoral immune responses is currently underway. Intensive study of LTNPs should shed valuable insight into the pathogenic mechanisms of HIV disease and hopefully will provide important information for the development of therapeutic and vaccine



strategies. (Fauci, Pantaleo, Menzo, Vaccarezza, Muro-Cacho, Graziosi, Cohen, LIR/NIAID; Multicenter AIDS Cohort Study Group; Montefiori, Duke Univ; Orenstein, George Washington Univ; Fox, Molecular Histolabs, Gaithersburg, MD; Pavlakis, FCRDC/NCI; Schwartzentruber, SB/NCI)

### International Studies on the Acquired Immunodeficiency Syndrome

AIDS is a global pandemic with over 18 million HIV-infected individuals worldwide. A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic and immunologic features of HIV-1 and HIV-2 infections in developing countries and in the U.S. In Pune, India, we established a prospective cohort of over 3,000 high-risk individuals attending sexually transmitted disease (STD) clinics, of whom 23% were seropositive for HIV-1. In a cohort of HIV-1 seronegative individuals followed over 15 months, the HIV incidence was 12.2% per year with rates as high as 28.6% for commercial sex workers. Recurrent genital ulcers and non-ulcerative STDs were independently associated with 11 and 19-fold increased risk of HIV seroconversion, respectively. Phylogenetic analysis of viral strains isolated from these individuals indicate that both clade B and C strains are present with high-titer neutralizing antibody present against clade B isolates. Additional studies among patients presenting to the Johns Hopkins Emergency Department demonstrated a rise in seroprevalence to 12.5% among 2,000 patients. A seroprevalence of 6.6% was documented by rapid HIV testing performed in the Emergency Department among patients of unknown serostatus. In perinatal studies in Zaire, Haiti, Malawi, and the U.S., we estimated a 28% perinatal transmission rate, of whom 31% may have been infected in utero, 58% intrapartum, and 11% postnatally utilizing HIV culture and PCR to determine timing of infection. To document transmission via breast feeding, we demonstrated HIV DNA, p24 antigen, and HIV by culture in 50-70% of breast milk specimens from HIV seropositive women within the first week following delivery. In a multi-center study comparing foscarnet and ganciclovir for the treatment of cytomegalovirus retinitis, we demonstrated a significant decline in HIV-1 viral load in both treatment arms. This anti-viral effect on HIV-1 replication appeared to be due to a significant effect primarily on cytomegalovirus replication. In studies of HIV-1 and HTLV-1 co-infection in Brazil, we documented a higher incidence of myelopathy and peripheral neuropathy among dually infected individuals. In addition, T- and B-cell responses to pneumococcal and tetanus vaccine immunizations were markedly diminished among dually infected individuals compared to those infected with HIV-1 alone. Additional studies are planned to further elucidate the immunologic modulations of HIV infection by co-infection with HTLV in these cohorts. (Quinn, Fauci, LIR/NIAID; Bollinger, Rompalo, Zenilman, Halsey, Kelen, Bartlett, Johns Hopkins, Baltimore, Maryland).

### Anti-Retroviral Therapy of HIV-1 Infection

An effort was directed toward studying the preventive and therapeutic aspects of HIV infection and the AIDS. A randomized trial comparing therapy with ZDV versus IFN- $\alpha$  versus the combination in 180 patients with early HIV-1 infection was completed and is being analyzed. A randomized, dose escalation study of U-90152S in combination with ZDV and ddI versus ZDV and ddI alone completed accrual of 87 patients and continues to generate data. Responsiveness was related to the preexistence or development of mutations in the reverse transcriptase gene. A dose-





escalation study of PMEA in 15 patients was completed and is being analyzed. A phase I study of CD4-Pseudomonas Exotoxin (sCD4-PE40) in HIV-infected patients determined the pharmacokinetics, maximally tolerated dose, and lack of preliminary efficacy of this agent. Measurements of plasma HIV RNA levels were found to be of value in the monitoring of patients with HIV infection. (Polis, Lane, Davey, Walker, Falloon, LIR/NIAID; Masur, Kovacs, Spooner, CC/NIH; Nussenblatt, Whitcup, NEI/NIH, Urdea, Kolberg, Eastman, Chernoff, Chiron Corp; Salzman, Baseler, Natarajan, Dewar, SAIC)

### Clinical Trials for the Prevention and Treatment of HIV-Associated Infections

An intensive effort directed at improving the prevention and treatment of AIDS-associated opportunistic infections continues. A phase I study of levofloxacin, an investigational quinolone, demonstrated that serum concentrations appropriate for the treatment of tuberculosis can be attained with safe, tolerable intermittent high-dose regimens. A study of pharmacokinetic drug interactions between stavudine, an anti-retroviral agent, and rifabutin or clarithromycin, drugs useful for the treatment or prevention of disease caused by *Mycobacterium avium-intracellulare*, has begun. An infrastructure for the collection of specimens from patients with tuberculosis has been established and assays for the detection and quantitation of *M. tuberculosis* in clinical specimens are being assessed. A study of the efficacy and pharmacokinetics of the investigational suspension formulation of atovaquone for the treatment of pneumocystis pneumonia has demonstrated a pharmacokinetic advantage of the suspension over the tablet formulation. A study of sulfasim, or CI-0694, a novel compound that can suppress and prevent antigen-specific antibody responses to sulfamethoxazole in animals, has just begun. This agent may prove useful in ameliorating the toxicity of trimethoprim-sulfamethoxazole and thus improve the treatment and prevention of pneumocystis pneumonia in some patients. A study of a ganciclovir-releasing intraocular implant with oral ganciclovir and a HIV-1 protease enzyme inhibitor is in the final stages of design. The assessment and follow-up of persons with idiopathic CD4<sup>+</sup> T-lymphopenia (ICL) in order to investigate pathogenesis and natural history is continuing. Finally, an outreach effort for the enrollment of women and members of minority and medically under-served populations in clinical trials has begun which includes seeing patients in a local community health center as well as establishment of a city-wide AIDS clinical trials information center. (Falloon, Lane, Davey, Polis, Walker, Sneller, LIR/NIAID; Masur, Kovacs, Spooner, Piscitelli, Cartwright, CC/NIH); Manischewitz, CBER/FDA; Whitcup, Nussenblatt, NEI/NIH)

### Immunologic Approaches to the Therapy of HIV Disease

An intensive effort was directed toward studying the potential therapeutic aspects of immunologic approaches to HIV infection. IL-2 therapy resulted in sustained increases in numbers of CD4<sup>+</sup> T cells and decreased expression of activation markers on CD8<sup>+</sup> T lymphocytes. The probability of manifesting these immunologic responses was shown to be directly associated with baseline CD4<sup>+</sup> T-cell count. Transient and consistent increases in viral load at the end of each infusion were noted in patients with early disease, while more sustained increases were noted in patients with advanced disease. Attempts to block cytokine induction by IL-2 with pentoxifylline *in vivo* failed to produce a clinical benefit or alterations in IL-2-induced TNF production. A study of IL-2 in patients with Kaposi's sarcoma was continued, in order to attempt to correlate immunologic



and clinical responses. A randomized controlled trial comparing IL-2 plus nucleoside analogues to nucleosides alone was completed; a second randomized controlled multicenter trial comparing 3-, 4-, and 5-day infusions of IL-2 plus nucleosides to nucleosides alone was continued. A dose escalation trial evaluating the safety and immunologic activity of subcutaneously administered IL-2 was expanded to include patients with less advanced disease and to compare different doses and dosing intervals. Toxicities of IL-2 administered subcutaneously were identical to those seen with intravenous IL-2, but generally less severe. A study evaluating the immunologic and antiviral activity of intravenous IL-2 in combination with an inhibitor of HIV-1 protease was initiated. Significant increases in CD4<sup>+</sup> T-cell counts and declines in viral load have occurred in most recipients of daily protease inhibitor with or without IL-2. A study was begun to determine whether timing IL-2 therapy based on *in vitro* correlates of immune activity produces greater and more durable responses than administering IL-2 on a fixed regimen. Studies evaluating the safety and activity of an anti-TNF antibody and a soluble TNF receptor were completed. Antibody administration consistently produced temporary declines in immunoreactive TNF levels; however, receptor administration was associated with increased TNF levels. Neither product had appreciable immunologic or antiviral activity *in vivo*. A study comparing IL-2 administration alone to IL-2 combined with either anti-TNF antibody or thalidomide was begun. A study generating random recombinatorial libraries of human immunoglobulin genes from HIV-infected individuals was continued. An anti-gp120 antibody was identified and is being prepared for clinical development. A study evaluating the survival and distribution of adoptively transferred, genetically marked, syngeneic lymphocytes was undertaken; cells containing the marker gene continue to be detected in the peripheral blood of all 6 recipients from 28 to 48 weeks post-transfer. A gene therapy study was begun testing the safety and activity of repeated infusions of syngeneic CD8<sup>+</sup> T cells engineered with a chimeric CD4- $\zeta$  TCR receptor. Four patients have received from 10 to 108 engineered cells without adverse effects. (Walker, Lane, Davey, Falloon, Polis, Sneller, LIR/NIAID; Masur, Kovacs, Spooner, Piscatelli, Leitman, Carter, CC/NIH); Blaese, Muul, Morgan, NCHGR/NIH; Baseler, Natarajan, Dewar, Salzman, SAIC, Frederick, MD; Koenig, MedImmune, Gaithersburg, MD; Burton, Scripps Institute, LaJolla, CA; Fyfe, Chiron Corporation, Emeryville, CA)

#### Investigation of the SCID-hu Mouse Model of HIV-1 Infection

The SCID-hu-PBL model was modified to allow direct reconstitution of SCID mice with PBL from HIV-1-infected donors rather than through secondary infection by subsequent challenge with purified viral stocks. The rationale for this modification was to enable better simulation of natural infection by transferring critical elements of the human immune system together with host-adapted virus. Intraperitoneal injection of harvested mononuclear cells from infected patients in this "hu-HIV/PBL-SCID" model led to successful engraftment in 84% of mice and caused no decrease in the number of human mononuclear cells harvested by peritoneal lavage relative to control mice engrafted with uninfected cells. Virus was readily detected in 98% of engrafted mice. Viremia was first detected in serum by quantitative PCR on day 7 and persisted through day 17. Proviral DNA nucleotide sequences from peritoneal lavage cells recovered from hu-HIV/PBL-SCID mice on day 17 were not significantly changed from those derived from donor PBL at the time of injection. Reconstituted hu-HIV/PBL-SCID mice that were untreated sustained a 75% decrease in human CD4<sup>+</sup> T lymphocyte recovery in peritoneal wash relative to control mice reconstituted with cells from healthy individuals. Treatment with FddA both significantly reduced CD4<sup>+</sup> T-cell depletion and inhibited viral recovery. Finally, whereas supplementation with high-titer anti-HIV human IgG had



no effect upon either CD4<sup>+</sup> T-cell recovery or viral isolation, treatment with anti-TNF monoclonal antibody favorably influenced both of these parameters. These data support the utility of the hu-HIV/PBL-SCID model in studying antiretroviral and immune-based approaches to treatment of HIV-1 infection. (Lane, Connors LIR/NIAID; Baseler, SAIC, Frederick, MD)

### Mechanisms of Cell Death in HIV and Other Retroviral Infections

Studies of T-cell lines expressing HIV envelope proteins combined with studies of T-cell lines infected with the LAI strain of HIV-1 were able to demonstrate that the killing of CD4<sup>+</sup> T lymphocytes that occurs in HIV infection appears to be mediated by a form of programmed cell death (PCD) that arrests cells at the G2/M interface of the cell cycle. Cells killed in this fashion demonstrate an abundance of a hyperphosphorylated form of the cyclin dependent kinase p34cdc2 and increased levels of cyclin B. Overexpression of bcl-2 was not observed as a compensatory mechanism and cell lines transfected to express large amounts of bcl-2 were still killed by HIV-mediated PCD suggesting that the mode of PCD seen in the setting of HIV infection is quite distinct from that seen in the setting of negative selection. These differences raise the possibility that one may be able to identify potential therapeutic candidates capable of specifically inhibiting the pathway of HIV-mediated PCD. (Cohen, Lempicki, Kolesnitchenko, Lowry, Lane, LIR/NIAID).

### Immunoregulation of Human Lymphocyte Function

B lymphocytes are a crucial cell in the response to infectious diseases. Our studies are directed at understanding the cellular and molecular mechanisms regulating B-lymphocyte activation, proliferation, and differentiation. The cellular studies have focused on the various cytokines necessary for efficient B-cell growth and differentiation while the molecular studies have focused on key proteins important in B-cell function. Some of these studies have led to findings relevant to the function of other cell types besides B lymphocytes.

The identification of BL34, now termed RGP1, has led to the discovery of a gene family that shares two highly conserved regions called HR1 and HR2. Utilizing these conserved regions, evolutionarily conserved homologs have been identified in *C. elegans*, yeast (SST2), and fungi (flbA). These homologies are of particular interest since SST2 and flbA regulate developmentally important signal transduction pathways. SST2 impairs signal transduction through the mating pheromone response pathway. Remarkably, expression of four mammalian RGP family members in yeast also blunted the response to pheromone and partially complement a *sst2* mutation. Since the pheromone response occurs by activation of a signaling pathway that employs heterotrimeric G proteins and MAP kinase homologs, RGPs may have important roles as regulators of similar signaling pathways in mammalian cells. The presence of high levels of RGP1 in germinal center B cells implies the presence of a unique G-protein linked signal transduction pathway utilized by these cells.

The discovery of GC kinase has led to the first demonstration of a functional role for an STE20 homolog in mammalian cells. STE20 functions upstream of the MAP kinase module in the yeast pheromone response pathway. We have found that GC kinase specifically activates the stress activated protein kinase (SAPK) pathway in a fashion similar to the activation of the MAP



kinase pathway by STE20 in yeast. The presence of high levels of GC kinase in germinal center B cells implies that the SAPK pathway is active in those cells. The role of this pathway in the germinal center remains to be determined. In addition, we have isolated a GC kinase homolog that is expressed at high levels in T cells. Studies are in progress to determine if the GC kinase homolog also activates the SAPK pathway.

CD22 is a B lymphocyte specific membrane protein that mediates cell-cell interactions. Besides its role as an adhesion molecule CD22 has a potent signal transduction capability. Within its intracellular domain is an ITAM-like motif that, following tyrosine phosphorylation, recruits several key downstream signaling molecules. Among the other known B-cell membrane proteins, only the intracellular portions of the mb-1 and B29 molecules contain ITAM motifs. Cross linking CD22 results in B-cell proliferation and greatly augments responses to other B-cell activation signals including CD40, Ig cross linking, and cytokines. In addition, Ig cross linking leads to CD22 tyrosine phosphorylation and recruitment of downstream effectors suggesting a complex interaction between the Ig receptor complex and CD22. Since CD22 is not expressed on the cell surface until B cells acquire IgD, it likely plays a role in augmenting responses to B-cell activation signals. Current studies include mapping of the sites within the intracellular domain that interact with downstream effectors, expression of dimerization constructs containing the intracellular portion of CD22 in cell lines and transgenically, and transgenic expression of human CD22 under the control of the IgH enhancer to determine if early expression of CD22 impairs B-cell development.

Our studies of the regulatory regions of B lineage-specific genes revealed more complexity than we anticipated. Each of the promoter regions is unique and utilizes distinct combinations of transcription factors. A critical PU.1 site was identified in the Bruton's tyrosine kinase promoter although binding sites for two other ets proteins and a SP-1 site have been found. The CD22 promoter has a strong *in vivo* footprint over a G rich sequence that binds an unknown transcription factor. This region is critical for the function of the CD22 promoter. Several important cis-elements have been identified in the CD19 promoter including a SP-1 site and a BSAP site that are *in vivo* footprints. An important TG rich and AT rich sequence have also been identified. A CD19 deficient cell line that also lacks BSAP has been created. Attempts to reconstitute BSAP in this cell line to determine if CD19 is restored is in progress.

BSAP is a critical transcription factor for B-cell development. We have identified a common BSAP splice product that deletes exon two resulting in a deletion in the DNA binding domain. This construct behaves like a dominant negative implying that BSAP interacts with another protein. We have also found that the particular context in which a BSAP cis-element is located determines how effectively BSAP transactivates. This also argues that BSAP interacts with another protein to modulate its activity. Antibodies have been made against the n-terminus and c-terminus of BSAP allowing the identification of the BSAP protein in cell lines and primary B cells. A construct for expressing BSAP transgenically has been made to test the hypothesis that BSAP down regulation is required for terminal B-cell differentiation. A BSAP targeting construct is in progress to delete BSAP from cell lines to better understand the role of BSAP in mature B-cell function.

Within germinal centers B cells are selected for the expression of high affinity antigen receptors. Those B cells with inappropriate or low affinity receptors face elimination via PCD. A likely candidate for mediating germinal center B-cell rescue has been bcl-2. However, bcl-2 is predominantly expressed in mantle zone B cells and not in germinal center B cells where selection





is occurring. We localized bcl-x, a bcl-2 related protein, within cells in the germinal center via immunocytochemistry and in a population of B cells enriched for germinal center B cells (centrocytes) that lacked significant levels of bcl-2. Furthermore, the levels of bcl-x rapidly increased following stimulation of B cells with CD40 a known stimulus that rescues germinal center B cells. These studies implicate bcl-x as the major mediator of the rescue of germinal center B cells undergoing affinity maturation in the germinal center.

Homeodomain containing proteins play crucial roles during embryonic development and in certain adult tissues. The HB9 gene encodes for a diverged homeodomain protein that is expressed at high levels in hematopoietic progenitors, germinal center B cells, developing motor neurons, and developing brain. We isolated the murine equivalent of the HB9 and characterized its genomic structure. We introduced a targeting construct for creating an HB9 null mouse into embryonic stem cells and are screening for homologous recombinants. This study should provide insights into the function of HB9 in embryonic and adult tissues.

These studies have led to a better understanding of normal B-lymphocyte function and have identified several families of proteins that likely play crucial regulatory roles in other tissues. They eventually should provide the basis for deciphering immune abnormalities and for manipulating certain signal transduction pathways and gene regulatory circuits in B lymphocytes. Such manipulation will allow a more sophisticated approach to immune modulation (Druey, Kang, Himmelmann, Riva, Zhang, Fauci, Kehrl LIR/NIAID/NIH; Tedder, Duke University; O'Shea, NCI; Blumer, Washington University; Kyriakis, Harvard University; Katz, Georgetown University).

#### Mechanisms Underlying Immune activation: signal transduction and the induced genetic response of T cells.

Immune activation results in the induced expression of many genes in the responding immune T cells. These genes are essential for expression of the differentiated phenotype and for proliferation of the T cells. Characterization of immediate-early induced genes has yielded significant insights into the process of cellular immune activation because these genes encode a variety of pleiotropic regulators. Induced expression of the genes generates the ability in these cells to respond and interpret many environmental cues and to make fundamental decisions, such as whether to die by apoptosis or to proliferate. To better understand the actual targets of the immediate-early genes, many of which encode transcription factors, and to establish a connection to ultimate biologic effects, we have initiated a long-term project to clone and characterize genes induced at later times after stimulation of primary human T cells. We have made significant progress and are beginning to clone full-length cDNA clones for several previously unknown genes. Ultimately this research will lead to a fundamental understanding of the cascades of events which are initiated by antigenic or mitogenic stimulation of T cells. Furthermore, new, potential targets for immunomodulatory therapies are likely to emerge from these studies.

Activation of T cells can also be effected by the HTLV I-encoded Tax protein. This protein is primarily responsible for the HTLV I-mediated transformation of T cells. One target of Tax essential for activation and transformation is the transcription factor NF- $\kappa$ B. This transcription factor is central also to antigen- or mitogen-induced expression of many immune-responsive genes. We have investigated how Tax activates NF- $\kappa$ B. We have ruled out many prior theories and have



determined that Tax can induce rapid turnover of the inhibitor of NF- $\kappa$ B, the I $\kappa$ B- $\alpha$  protein, leading to activation of the transcription factor. Significantly, Tax appears to induce this turnover by mimicking a step in the signaling pathway which is also activated by extracellular stimulations; both means of activation have the same requirement for site-specific and signal-dependent phosphorylation of the I $\kappa$ B- $\alpha$  inhibitor. Tax may therefore activate an early pleiotropically-acting signaling protein, which may explain, at least in part, the many effects which have been ascribed to the Tax protein in cells.

We have investigated the signaling paths which originate with the T-cell receptor or which originate with TNF stimulation. Both stimuli will potentially activate the NF- $\kappa$ B transcription factor. We determined that T-cell receptor-mediated activation of this transcription factor requires synergistic interactions between the Raf oncoprotein, a kinase, and the Ca-activated phosphatase calcineurin. This result is consistent with the ability of the calcineurin-inhibiting immunosuppressants cyclosporin A and FK506 to inhibit T-cell receptor-mediated activation of NF- $\kappa$ B. Signaling by TNF is distinct since neither the Ca signal nor the Raf kinase are involved. We have cloned a member of a family of proteins which mediate signaling through the TNF receptors by associating with their intracellular tails. We have found that one of these proteins can directly activate NF- $\kappa$ B when overexpressed in some T cells and we are investigating the underlying mechanism. (Siebenlist, Kanno, Lin, Ellinger, LIR/NIAID)

#### Mechanisms of Activation of NF- $\kappa$ B.

NF- $\kappa$ B is a primary regulator of genes encoding immunomodulatory proteins. In addition, NF- $\kappa$ B regulates the expression of HIV and its activation may be essential to establish a productive infection in cells. This transcription factor is rapidly activated in cells via inactivation of its inhibitor, I $\kappa$ B- $\alpha$ . The inhibitor normally associates with NF- $\kappa$ B and retains it in the cytoplasm, thus preventing the transcription factor from entering nuclei and inducing the expression of its target genes. We have demonstrated that inactivation proceeds via signal-induced phosphorylation of the inhibitor, immediately followed by its degradation. Contrary to prior dogma, we determined that phosphorylation of the inhibitor does not dissociate it from the transcription factor. This also indicated that proteolytic degradation of the inhibitor was required. We confirmed this by blocking the degradation of I $\kappa$ B- $\alpha$  with the anti-proteases called calpain inhibitors I and II; this blocked activation of NF- $\kappa$ B while having no effect on the signal-induced phosphorylation. Protease inhibitors such as calpain inhibitors may have therapeutic uses to inhibit inflammation or viral infections by blocking activation of NF- $\kappa$ B.

We have performed a systematic mutational analysis of the I $\kappa$ B- $\alpha$  protein to investigate a link between its phosphorylation and its degradation and to determine sites and regions relevant to function. Our analysis involved permanent transfection of the mutants into T cells, where the exogenously introduced protein could be shown to be subject to the same regulation as the endogenous inhibitor. This research allowed us to identify two closely spaced serines in the N-terminal portion of the inhibitor which become inducibly phosphorylated upon cellular stimulation and whose phosphorylation is necessary for subsequent proteolytic degradation. When phosphorylated at these sites, the I $\kappa$ B- $\alpha$  protein is tagged and in this way targeted for destruction. Preliminary evidence indicates that degradation proceeds via ubiquitination and digestion by



proteasomes. We will seek the identity of the I $\kappa$ B- $\alpha$  kinase and the molecular components needed to target ubiquitin-ligating enzymes to the tagged I $\kappa$ B- $\alpha$  protein. These proteins may provide therapeutic targets. Ultimately we intend to trace back to the membrane the signaling pathways which activate NF- $\kappa$ B. (Brown, Lin, Baldi, Siebenlist, LIR/NIAID)

#### Physiologic Functions of NF- $\kappa$ B/I $\kappa$ B Proteins.

NF- $\kappa$ B is a family of dimeric complexes, each composed of members of a family of Rel-related polypeptides. Similarly, there exists a family of I $\kappa$ B-related proteins. Individual members of these families have at least partly distinct functions. To investigate the unique physiologic roles of the NF- $\kappa$ B subunit p52 and of Bcl-3, an I $\kappa$ B family member, we have generated mice bearing targeted disruptions of the respective genes. This approach is especially useful in the case of Bcl-3, a protein whose physiologic role is quite obscure, but which can be shown to cause  $\kappa$ B-dependent transactivation by interaction with p50 or p52 homodimers in transfection experiments. To uncover defective phenotypes of the knock-out mice we are at present immunologically challenging these mice by viral infection.

We are generating transgenic mice overexpressing Bcl-3, p52 or p50 in B or T cells. These mice will be useful in determining functions for these proteins, complementing the knock-out studies. In addition, the transgenic mice will help in finding the mechanisms by which p52 and Bcl-3 can have oncogenic activities in human tumors where expression appears deregulated as a result of chromosomal translocation of their genes.

Another approach to understanding functional targets for NF- $\kappa$ B focuses on the action of this transcription factor in the context of promoters, i.e., in the context of other potentially interacting transcription factors. We discovered that the NF- $\kappa$ B subunits p65 and c-Rel can transactivate promoters by binding to the serum response factor (SRF). The synergistic interaction between these factors does not require a  $\kappa$ B binding site; p65 and c-Rel activate a latent transactivation domain in SRF. The near-ubiquitously expressed SRF is thought to mediate the induced expression of a large number of genes. Our discovery greatly expands the list of genes potentially affected by NF- $\kappa$ B.

Through an investigation of various subclones of U937 we found a correlation between the expression of seprocidin proteases and the inability to support good growth of HIV upon infection. Those cells lacking the expression of these proteases supported growth well. The presence of the seprocidins was discovered due to their ability to specifically truncate the p65 protein of NF- $\kappa$ B during cell extraction. (Franzoso, Siebenlist, Biswas, Poli, Fauci, LIR/NIAID)

#### Immunopathogenesis of *Chlamydia trachomatis* Infections"

Over the past decade studies have been in progress to define the clinical spectrum and transmission efficiency of *Chlamydia trachomatis* infection, to develop improved molecular amplification assays for diagnostic purposes, and to examine the pathogenesis of *C. trachomatis* and *C. pneumoniae* infection in patients with genital, ocular, respiratory, and cardiac disease. Following the development of PCR for *C. trachomatis* in our laboratory, we have been able to



demonstrate a marked increase in sensitivity of detection of chlamydial genital and ocular infections in patients attending STD clinics and in trachoma-endemic areas, respectively. In studies of over 10,000 patients, sensitivity and specificity of PCR for *C. trachomatis* was 91% and 99%, respectively, compared to culture in which sensitivity ranged from 60 to 75%. We further developed and evaluated a co-amplification PCR assay for both *C. trachomatis* and *N. gonorrhoeae*. Utilizing this technique in a variety of populations, we have demonstrated in Baltimore, Jamaica, Malaysia, the Philippines, and Uganda, chlamydia infections ranging from 10% to 33%. By applying molecular amplification in transmission studies of *C. trachomatis*, we have been able to demonstrate an equal bi-directional transmission efficiency rate of 68% for *C. trachomatis*. We have initiated a series of studies to examine the local production of cytokines in response to *C. trachomatis* infection among individuals in varying stages of trachoma in Tanzania. In patients with follicular and scarring trachoma, TGF- $\beta$  was the predominant cytokine produced and there was no evidence of IL-6 production. *C. trachomatis* infection in trachoma appears to induce a Th1 response locally characterized by TGF- $\beta$  production and subsequent development of corneal neovascularization or pannus.

In studies of *C. pneumoniae* infection, we have demonstrated *C. pneumoniae* infection in 8% of 700 patients with respiratory disease with rates as high as 33% in immunocompromised patients undergoing bronchoalveolar lavage. We have been able to propagate *C. pneumoniae* *in vitro* in pulmonary alveolar macrophages, pulmonary artery endothelial cells, human aortic endothelial cells human umbilical vein endothelial cells, and aortic artery smooth muscle cells. In an evaluation of the hypothesis that *C. pneumoniae* may be associated with atheromatous plaques in individuals with coronary heart disease, we have screened atheromas and/or coronary artery tissue from 50 patients with severe heart disease. Although the majority remain negative for *C. pneumoniae*, six patients had *C. pneumoniae* recovered by culture and demonstrated by PCR, electron microscopy, and *in situ* hybridization in the coronary atheromas, coronary artery endothelial cells, and surrounding tissue in six patients. These observations indicate that *C. pneumoniae* may be associated with atherosclerosis and that more studies of this relationship are warranted. (Quinn, LIR/NIAID; Gaydos, Viscidi, West, Zenilman, Rompalo, Johns Hopkins, Baltimore, MD)

#### Studies in Patients with Vasculitic Syndromes

We have continued our study of the efficacy and safety of low-dose methotrexate (MTX) in treating Wegener's granulomatosis (WG). Forty-two patients who did not have immediately life-threatening disease were studied. Glomerulonephritis was present in 21/42 (50%) of patients. The median follow-up was 33.7 months. Weekly administration of MTX and prednisone resulted in remission of disease in 34/42 patients (80%). The median time to remission was 4.2 months. Twelve patients did not achieve remission: three patients had progressive disease that required institution of cyclophosphamide therapy; five patients had clinical improvement but continued to exhibit signs of active disease; two patients developed MTX-induced pneumonitis prior to achieving remission; two patients died of opportunistic infections prior to achieving remission. Fifteen of the 34 patients achieving remission experienced a relapse of disease. The estimated median time to relapse for all patients achieving remission was 32 months. Ten patients who relapsed were treated with a second course of MTX plus prednisone. A second remission was induced in 8 out of 10 patients. MTX plus prednisone may be an acceptable alternative form of therapy for selected





patients with WG.

In preliminary studies we have used skin blisters induced by suction as a method to study the *in vivo* inflammatory response of patients with WG. We have shown that patients with active WG produce 50- to 100-fold higher levels of TNF- $\alpha$  in blister fluid when compared with normal controls. Such high TNF- $\alpha$  levels are not seen in blister fluid from patients with a variety of other inflammatory and infectious diseases. Levels of IL-1 $\beta$  and IL-6 were also significantly elevated in blister fluid from patients with active WG. These findings have important therapeutic implications since specific inhibitors of TNF- $\alpha$  and other pro-inflammatory cytokines are currently available and may be effective in the treatment of WG and related vasculitic syndromes. Based on these findings we have initiated a Phase I trial of the drug lisofylline in the treatment of patients with WG. Lisofylline is a compound that functionally blocks pathways of IL-1 and TNF- $\alpha$  signal transduction and has anti-inflammatory activity. (Sneller, Langford, Talar-Williams, Fauci, LIR/NIAID; Gallin, Hallahan, OSD/NIAID)



### Administrative, Organizational, and Other Changes

The major theme of the Laboratory of Immunoregulation (LIR) continues to be focused on studies of the immunopathogenesis of human immunodeficiency virus (HIV) disease with a particular emphasis on the host factors involved in disease pathogenesis. In addition, we have continued our therapeutic studies which are aimed predominantly at reconstitution of the immune system in HIV-infected individuals. The space, resources, and FTEs of the LIR have remained stable over the past year.

Drs. Andrew and Elaye Dayton have left the LIR to assume new positions at the FDA. Dr. Priscilla Biswas returned to Italy after a productive 3-year post-doctoral fellowship in the LIR and has joined the AIDS Immunopathogenesis Unit of the San Raffaele Institute in Milan, Italy. Dr. Mario Ostrowski has joined the LIR as a post-doctoral fellow following his Infectious Diseases Fellowship at the University of Toronto. Dr. Stephania Paolucci has joined the LIR for a post-doctoral fellowship from the University of Ancona, Italy.



### Honors, Awards, and Scientific Recognition

During the past year, members of the LIR have received numerous awards and honors.

Dr. Fauci continued to serve on a number of committees of scientific and administrative importance such as the International Steering Committee of the Tenth International Conference on AIDS, Yokohama, Japan. He is also Councillor in the Association of American Physicians.

Dr. Fauci serves on the Editorial Boards of *The New England Journal of Medicine*, *The Journal of Immunopharmacology*, *Journal of Biomedical Science*, *EOS*, *Cellular Immunology*, *AIDS Research and Human Retroviruses*, *AIDS Patient Care*, *Immunity*, and *Molecular Medicine*. Dr. Fauci is an advisory editor of *The Journal of Experimental Medicine*. He serves on the Advisory Boards of *Immunopharmacology* and *Immunotoxicology*, *Clinical Immunology* and *Immunopathology*, and the *Journal of Clinical Immunology*, and is on the Editorial Advisory Council of *La Ricerca in Clinica e in Laboratorio*. He continued his duties as associate editor of the *Journal of the Acquired Immunodeficiency Syndrome*.

Dr. Fauci continues as editor of Harrison's Principles of Internal Medicine; Transactions of the Association of American Physicians; Current Therapy in Allergy, Immunology and Rheumatology; and Harrison's Principles of Internal Medicine - Companion Handbook. He is an associate editor of Current Therapy in Internal Medicine and AIDS, Etiology, Diagnosis, Treatment, and Prevention. In addition, he is an editor along with Dr. John I. Gallin of Advances in Host Defense Mechanisms.

Dr. Fauci recently received the Doctor of Sciences, Honoris Causa, degree from Duke University.

This past year, Dr. Fauci was Plenary Speaker at the 10th International AIDS Conference and Conference on STDs, in Japan; the 43rd Annual Meeting of the American Society of Tropical Medicine and Hygiene, Cincinnati, OH; and at the Annual Meeting of the Catalan Society of Internal Medicine, Barcelona, Spain.

Dr. Fauci gave a State of the Art Lecture at the Second National Conference on Human Retroviruses and Related Infections, Washington, DC. He gave the prestigious Maurice Hilleman Annual Lectureship at Children's Hospital of Philadelphia; the Richard S. Farr Lectureship of the American Academy of Allergy and Immunology, New York, NY; the Theobald Smith Lectureship of the Albany Medical College, Albany, NY; and the First Annual David E. Rogers Memorial Grand Rounds, The New York Hospital-Cornell Medical Center, New York, NY.

Dr. Fauci was Keynote Speaker at the 10th World Congress on Gastroenterology, Los Angeles, CA; the 12th Annual Symposium on Nonhuman Primate Models for AIDS, Boston, MA; the Neuvieme Colloque des Cent Gardes, Paris, France; and the Clinical Immunology Society Workshop on HIV Immune-Based Therapies, Baltimore, MD. He was an Invited Speaker at Nature's International Conference on the Immune System: A Model Organ?, Boston, MA; the Symposium on Molecular and Genetic Immunoprobes for Biotechnology, the Annual Meeting of the American Association of Immunologists, "Immunopathogenic Mechanisms of HIV Disease," Atlanta, GA; and the First Annual Tim Gill Lectureship of the University of Colorado Health



Sciences Center, Denver, CO.

This past year, Dr. Fauci received the following awards: the National Italian American Foundation Award for Special Achievement in Medicine and Science; the Honorary Fellowship Award, American Academy of Allergy and Immunology; the Richard and Hinda Rosenthal Award of the American College of Physicians; the Thirty-Eighth Theobald Smith Award of the Albany Medical College; the Ernst Jung Prize for Medicine, Hamburg, Germany; the Ellis Island Medal of Honor for Medical Research; the Gold Medal of the Autonomous University of Barcelona, Spain; membership in the Royal Academy of Medicine of Barcelona; and Internist of the Year Award of the Catalan Society of Internal Medicine.

Dr. H. Clifford Lane was Invited Speaker, "Role of viral burden and immunologic status in deciding when to initiate anti-retroviral or immune based therapy," at the Tenth International Conference on AIDS, Yokohama, Japan; Distinguished Lecture Series Symposium Speaker, "Recent Advances in AIDS," at the American College of Physicians Meeting, Atlanta, Georgia; and Chairman, Minisymposium on HIV, Annual Meeting of the American Association of Immunologists, Atlanta, GA. He was Invited Speaker, "Immunologic Approaches to the Therapy of HIV Infection," at the Third International Symposium on Clinical Immunology, San Francisco, CA.

Dr. Thomas Quinn was elected to the Association of American Physicians. He was Plenary Speaker at the American Association for the Advancement of Science; at the International Conference on STDs and AIDS, Singapore; and at the International Conference on Chlamydial Infections, France.

This past year he was a Panel Member for the U.S.-Japan Cooperative Medical Science Program on HIV and Related Retroviruses; for the Food and Drug Administration Antiviral Review Committee; and for the Committee on Population, National Academy of Sciences (Working Group on Future Perspectives on the AIDS Epidemic in Africa). Dr. Quinn was a member of the Advisory Group to the CDC, NIH, and IDSA, Guidelines for the Prevention of Opportunistic Infections. Dr. Quinn was a member of the Advisory Committee on Epidemiology for the Office of AIDS Research. Dr. Quinn received the Charles C. Shepard Science Award for Outstanding Scientific Publication; the National Center for Infectious Diseases Special Recognition Award; the James H. Nakano Citation for Outstanding Scientific Publication. He was also Representative for North America, International Union Against Venereal Diseases and Treponematoses (IUVDT).

In addition, Dr. Quinn continues to serve on the Editorial Boards of ten journals related to STD, HIV, and infectious diseases, and on a number of Advisory Committees, including the Advisory Committee to review international programs for the Division of Microbiology and Infectious Diseases, NIAID.

Dr. Ulrich Siebenlist won the NIH Merit Award.

In the last year, Dr. Giuseppe Pantaleo was a keynote speaker at the Tenth International AIDS Conference, Yokohama, Japan; at the Pediatric AIDS Foundation Think Tank #11 on Immunopathogenesis of HIV Infection in Infants and Children, Santa Barbara, California; at the Workshop on 'Early Phases of HIV Infection, Rockville, Maryland; the Comprehensive Management of HIV Disease: HIV Speakers' Forum, V Annual Update Meeting, Palm Beach, Florida; the 6th





Convegno Nazionale di Immunologia and Allergologia Pediatrica, Brescia, Italy; the VIII Convegno Nazionale AIDS, Bologna; the Challenge in Virology, Saanen/Gstaad, Switzerland; Chairman and Speaker at the 2nd National Conference Human Retroviruses and Related Infections, Washington D.C.; the Gordon Research Conference - Chemotherapy of AIDS, Ventura, California; Seminar at Institut Paoli Calmettes, INSERM U.119, Marseille, France; the VIII Mediterranean Symposium On HIV Infection, Toulon, France; a Seminar at Ciba-Geigy, Basel, Switzerland; the International Symposium on Combination Antiretroviral Therapy for HIV Infection, Berlin, Germany, 25-26 March, 1995; The International AIDS Society - Advanced Course in Pathogenesis, Antiretrovirals, and Selected Populations with HIV, Chicago, Illinois; Symposium on AIDS: Therapeutic and Prophylactic Challenges, Hood College, Frederick, Maryland; Workshop on IL-12 in Infection, The Cloister, Bethesda, Maryland.

Dr. Sharilyn Stanley was Guest Lecturer, "HIV: The Epidemic, the Virus, and the Immune Response," HIV/AIDS in Native American Communities, Indian Health Service/OAR, Albuquerque, New Mexico; Guest Lecturer, "Primary Disease Due to Human Retroviruses: HIV-1, HIV-2," Advances in Diagnostic Pathology of Infectious Diseases. AFIP, Washington, D.C. She was also Invited speaker, "AHI/AIDS Update: 1995, Today's Challenges," the Third International Congress on Biological Response Modifiers, Cancun, Mexico; and Invited speaker, "New Insights into the Immunopathogenesis of HIV Disease," the "UV, HIV, and AIDS" Symposium, the Annual Meeting of the American Society for Photobiology (ASP), Washington, D.C.

Dr. Michael Polis was promoted to Associate Clinical Professor of emergency medicine at the George Washington University Medical Center.

Dr. Judith Faloon won the NIH Merit Award, and is Vice-President, Greater Washington Infectious Diseases Society.

Dr. John Kehrl won the Outstanding Service Medal, PHS

Dr. Drew Weissman won the NIH Merit Award. He was an invited speaker at a plenary session: Dendritic Cells: Antigen Presenting Cells of T and B Lymphocytes. Keystone Symposium, Keystone, CO.

This past year, Dr. Audrey Kinter won the NIH Merit Award.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00700-02 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Virologic and Immunologic Events Associated with Primary HIV Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

PI: G. Pantaleo Visiting Scientist LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID

M. Vaccarezza Visiting Fellow LIR, NIAID

C. Graziosi Visiting Scientist LIR, NIAID

S. Paolucci Visiting Fellow LIR, NIAID

COOPERATING UNITS (if any)

IRCM, Montreal (R. Sekaly); H. Soudeyns); PRI, Frederick, MD (J. Adelsberger); U. Alabama, Birmingham (G. Shaw, M. Saag); Regional Primate Ctr, Tulane, LA (L. Martin)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have demonstrated high levels of human immunodeficiency virus (HIV) DNA and RNA synthesis in peripheral blood mononuclear cells during primary HIV infection. Dramatic downregulation of the levels of HIV RNA synthesis coincides with the emergence of HIV-specific immune responses. There is a discrepancy, however, between the downregulation of viremia and the minimal or lack of detection of changes in the levels of HIV DNA in mononuclear cells. In the simian immunodeficiency virus (SIV) model numerous individual infected cells were localized in peripheral lymph nodes of monkeys as early as day 7 post-inoculation. Trapping of virions in the follicular dendritic cell (FDC) network was initially detected at week 2 post-inoculation and progressively increased over time, whereas the number of SIV-infected cells decreased. Trapping of virions in the FDC network was temporally associated with a rise in the levels of complement-binding antibodies. Similar kinetics of HIV distribution in lymph nodes have been observed in individuals with primary HIV infection. We have demonstrated major expansions in T cells manifesting a restricted set of V $\beta$  families during the primary immune response to HIV. Cells expressing the expanded V $\beta$ s were predominantly CD8<sup>+</sup> T lymphocytes, and were activated. These expanded CD8<sup>+</sup> T-cell subsets were involved in the expression of cytokines, and mediated specific cytotoxic activity against HIV envelope was detected within the expanded cell populations. Nucleotide sequences of recombinant clones of the expanded V $\beta$ s demonstrated the oligoclonal (i.e., antigen-specific) nature of these expansions. We have demonstrated that the expansion of these V $\beta$  families was driven by HIV antigens. Three patterns of V $\beta$  expansions were observed. These different patterns appear to be associated with different clinical outcomes.



Cooperating Unites (Continued)

Molecular Histology, Gaithersberg, MD (C. Fox); DAIDS, NIAID (S. Schnittman, C. Diffenbach, R. Black, P. Sager); San Raffaele Inst, Milan, Italy (G. Tambussi, A. Lazzarin); U Geneva (L. Perrin); U Washington (L. Corey); NINDS (W. Biddison).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00676-03 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunopathogenic Mechanisms of HIV Infection: The Role of Cellular Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: G. Pantaleo Visiting Scientist LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID

C. Muro-Cacho Visiting Associate LIR, NIAID

M. Vaccarezza Visiting Fellow LIR, NIAID

COOPERATING UNITS (if any)

George Washington U (J.Orenstein); Mol Histolabs, Gaithersburg, MD (C.Fox); PRI, Frederick, MD (M.Baseler); Tulane U (L.Martin); NIAID (C.Dieffenbach, R.Black, P.Sager)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

One of the major characteristics of human immunodeficiency virus (HIV) infection is the intense degree of cellular activation that occurs throughout the course of HIV disease. Cellular activation plays a major role in the pathogenesis of HIV disease since HIV replicates much more efficiently and spreads more readily among activated cells. In addition, persistent immune activation contributes to the immune defects in HIV infection by inducing energy and/or apoptosis among activated cells. We have investigated the immunopathogenic mechanisms responsible for CD4<sup>+</sup> T-cell depletion in HIV infection and the role of cellular activation in this process. Apoptosis intensity is 3- to 4-fold higher in lymph nodes of HIV-infected individuals compared to those of HIV-uninfected individuals. Generally, all compartments of the lymph node are involved in the apoptosis phenomenon in HIV-infected lymph nodes. Apoptosis correlates with the degree of activation of the lymphoid tissue and the chronic immune activation associated with HIV infection. In late stages of HIV disease, apoptosis occurs with similar intensity but in different compartments. Apoptosis does not correlate with progression of the disease (i.e., CD4<sup>+</sup> T-cell count) or with viral burden. All lymphocyte subsets (B and T cells, CD4<sup>+</sup> and CD8<sup>+</sup>) have been shown to undergo apoptosis. During primary HIV infection, apoptosis correlates with the degree of activation of the lymphoid tissue but not with viral burden. The importance of cellular activation in HIV infection is underscored by the observation that cyclosporin A (CsA), inhibits HIV infection in vitro (60% to 80%). CsA has been shown to modulate a series of parameters during primary infection in SIV-infected monkeys. CsA administration delays onset of viremia, prevents a drop in CD4<sup>+</sup> T cells and an increase in CD8<sup>+</sup> T lymphocytes, increases antibody response, and decreases apoptosis intensity in lymph node.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00678-03 LIR

## PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Exogenous Activation of the Immune System in the Pathogenesis of HIV Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.K. Stanley Senior Clinical Investigator LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID

M. Ostrowski Visiting Fellow LIR, NIAID

## COOPERATING UNITS (if any)

Molecular Histolabs, Gaithersburg, MD (C.Fox); G. Washington Univ. (J.Orenstein); PRI, Frederick, MD (M.Baseler); ACB/NIAID (P.Golway); Mass. Gen. Hosp (M.Sykes)

## LAB/BRANCH

Laboratory of Immunoregulation

## SECTION

Immunopathogenesis Section

## INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

## TOTAL STAFF YEARS:

4.0

## PROFESSIONAL:

2.0

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ © Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is directed at delineating the pathogenic mechanisms of human immunodeficiency virus (HIV) infection and the role of immune activation in the propagation of disease and destruction of the immune system. We have previously demonstrated that HIV infects the human thymus in the SCID-hu mouse and leads to depletion of thymocytes and destruction of the microenvironment. Treatment of SCID-hu mice with cyclosporin A blocks infection of the thymus, and this inhibition appears related to a decrease in cellular activation. Activation of the human immune system in vivo appears to accelerate the course of HIV disease. We demonstrate that, after tetanus toxoid booster inoculation of HIV-infected individuals, HIV can be more readily isolated from their peripheral blood mononuclear cells (PBMCs) in vitro and plasma viremia increases over 2 to 3 weeks. The degree of increase in in vitro virus expression and viremia appears to correlate somewhat with total CD4 lymphocyte count and also with the ability of the individual to respond immunologically to the tetanus boost; thus the more immunologically intact HIV-infected individuals mount a more appropriate immune response to tetanus and thereby experience a more dramatic increase in viremia and in vitro isolation of HIV from their PBMCs. HIV seronegative individuals become more susceptible to in vitro infection of their PBMCs with HIV after tetanus toxoid booster immunization. This heightened susceptibility to in vitro infection occurs at the time that the individual is making a vigorous response to tetanus as assessed by PBMC proliferation to tetanus in vitro and production of tetanus specific immunoglobulin.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00720-01 LIR

## PERIOD COVERED

October 1, 1994 to September 30, 1995

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Mycobacteria Tuberculosis in the Pathogenesis of HIV Disease

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Goletti Guest Researcher LIR, NIAID

Others: D. Weissman Senior Staff Fellow LIR, NIAID

A.S. Fauci Chief LIR, NIAID

K.M. Roche Study Coordinator LIR, NIAID

## COOPERATING UNITS (if any)

Montefiori Medical Center (R. Klein, S. Munsiff); Johns Hopkins Univ (N. Graham);  
Catholic Univ of Rome, Italy (L. Ortona, R. Cauda)

## LAB/BRANCH

Laboratory of Immunoregulation

## SECTION

Immunopathogenesis Section

## INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

## TOTAL STAFF YEARS:

1.2

## PROFESSIONAL:

1.2

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ © Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mycobacteria tuberculosis (MTB) is a worldwide infection whose prevalence has increased in part due to HIV infection. Epidemiological data have demonstrated that HIV-infected individuals are more susceptible to MTB infection and disease. The purpose of this study was to delineate how MTB modulates HIV infection *in vivo* and in *in vivo* models. We measured plasma viral load, a direct reflection of lymphoid viral replication, in HIV-infected individuals before, during, and after active MTB disease. It has been observed that the plasma viral load of HIV-infected individuals is increased during the acute phase of MTB disease compared to before the onset of the disease and after treatment. To evaluate the mechanisms involved in MTB-induced HIV replication, we studied the virologic and immunologic responses induced by MTB and the constituent antigen PPD in an *in vitro* system using primary PBMC and lymph node cells isolated from HIV-infected individuals. The data demonstrated that MTB induced HIV replication *in vitro* in CD8 depleted lymphocytes of HIV-infected individuals with a history of PPD positivity in the absence of exogenous stimulation. The increase of MTB- or PPD-mediated increase in HIV production correlated with the level of cellular activation as demonstrated by an expansion of CD4<sup>+</sup>, CD25<sup>+</sup> cells and by an increase in cellular proliferation. We have also demonstrated that MTB and PPD increased viral replication in an acute infection model where PBMC from healthy donors with positive skin tests for PPD have been infected with HIV-1 primary isolates, and this effect was correlated with the level of cellular activation and proliferation. In conclusion MTB increased viral replication *in vivo* and in an *in vitro* model. This MTB-mediated viral production likely occurs through the activation and infection of responding T cells. We believe that these findings may be important to further delineate the immunopathogenic mechanisms of HIV disease and to develop therapeutic strategies based on a knowledge of these mechanisms.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00699-02 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cytokine Expression in HIV Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Graziosi Visiting Scientist LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID  
G. Pantaleo Visiting Scientist LIR, NIAID  
K.R. Gantt Biologist LIR, NIAID  
J.F. Demarest Biologist LIR, NIAID  
O.J. Cohen Medical Staff Fellow LIR, NIAID

COOPERATING UNITS (if any)

IRCM, Montreal (R.P. Sekaly, J.P. Fortin)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ © Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously demonstrated that cytokines play a major role in the pathogenesis of HIV disease both in the modulation of HIV expression and in the immunologic abnormalities noted in HIV-infected individuals. We have investigated the constitutive expression of a panel of cytokines including interleukin (IL)-1 $\beta$ , IL-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)- $\alpha$ , and interferon (IFN)- $\gamma$  in peripheral blood (PB) and lymph nodes (LN) from human immunodeficiency virus type 1 (HIV-1)-infected individuals during different stages of disease. Constitutive expression of IL-1 $\beta$ , IL-10, IFN- $\gamma$ , and TNF- $\alpha$  in PB and LN is significantly higher in HIV-infected individuals compared to HIV-negative individuals. Levels of IL-6 increase in PB with disease progression and levels of IL-2 and IL-4 are low to absent at any stage of disease. Longitudinal analysis of constitutive cytokine expression in PB of HIV-infected individuals at different stages of disease has been unable to demonstrate changes in the pattern of cytokine expression associated with disease progression. Constitutive cytokine expression in sorted CD4 $^{+}$  and CD8 $^{+}$  T lymphocytes has shown that CD4 $^{+}$  T cells express very low levels of cytokines at any stage of infection. CD8 $^{+}$  T cells are involved in the constitutive expression of IFN- $\gamma$ , and to a lesser extent of IL-10. Stimulation in vitro of purified CD4 $^{+}$  T cells from patients during different stages of disease has not demonstrated any change in the pattern of cytokine expression associated with disease progression. Longitudinal analysis of constitutive cytokine expression in PB from HIV-infected individuals following primary infection has shown that levels of IL-2, IL-4, and IL-6 are low to absent in PB as early as the period of primary infection. In contrast, high levels of constitutive expression of IL-10, IFN- $\gamma$ , and TNF- $\alpha$  are associated with primary HIV infection.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00677-03 LIR

## PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Cytokines In the Regulation of HIV Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Kinter Biologist LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID

D. Goletti Guest Researcher LIR, NIAID

## COOPERATING UNITS (if any)

LID/NIAID (S. Bende); LIR/NIAID/CC (H.C. Lane, B. Baird); DAIDS (L. Fox); Molecular Histology, Gaithersburg, MD (C. Fox) CABM.UMDNJ, RWMS (A.B. Rabson)

## LAB/BRANCH

Laboratory of Immunoregulation

## SECTION

Immunopathogenesis Section

## INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

## TOTAL STAFF YEARS:

2.5

## PROFESSIONAL:

2.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cellular and molecular pathways involved in the regulatory effects of proinflammatory and immunoregulatory cytokines on human immunodeficiency virus (HIV) expression/replication were investigated. The central role of endogenous proinflammatory cytokines, particularly interleukin (IL)-1 $\beta$  and tumor necrosis factor (TNF)- $\alpha$ , in driving HIV replication in acutely infected peripheral blood mono-nuclear cells (PBMC) stimulated with IL-2 was demonstrated. Various physiologic cytokine inhibitors were found to suppress HIV replication in this system by interfering with the autocrine/paracrine loop of cytokine mediated stimulation of HIV production. Bacterial lipopolysaccharide (LPS), a physiologic stimulator of proinflammatory cytokine production by monocyte cells, was shown to induce HIV expression in a chronically infected monocytic cell line (U1) and this effect was demonstrated to be dependent upon the stimulation of an autocrine/ paracrine loop involving endogenous IL-1 $\beta$ . Anti-proinflammatory cytokines, such as transforming growth factor (TGF)- $\beta$ , IL-4, and IL-13, and IL-1 receptor antagonist (ra), inhibited LPS-induced HIV expression primarily by suppressing IL-1  $\beta$  and increasing IL-1ra production. Proinflammatory cytokines were also evaluated for their effect on ex vivo HIV replication. IL-1-  $\beta$ , IL-6 and TNF- $\alpha$  were found to enhance HIV production by MC of certain HIV-infected subjects. Cross-linking of CD30 on the surface of HIV-infected cells by CD30-CD30 ligand interactions induced HIV expression by a TNF-independent, NF- $\kappa$ B dependent pathway. The ability of various immunoregulatory cytokines, such as interleukin (IL)-2 and IL-12, were evaluated with regard to their relative stimulatory effects on HIV production by CD4<sup>+</sup> T cells versus their ability to stimulate CD8-mediated nonlytic viral suppressor (CD8s) activity in lymph node (LN) and PBMC from HIV-infected subjects. IL-2 potentially stimulated CD8s, an effect which overrode its inductive effect on HIV production, resulting in an inability to isolate virus in the presence of CD8<sup>+</sup> cells. Other immunoregulatory cytokines, such as IL-12, were not as effective in inducing CD8s and thus allowed viral replication in the presence of CD8<sup>+</sup> cells. PBMC from HIV-infected subjects undergoing IL-2/AZT combination therapy are being analyzed for evidence of IL-2 induced increases in CD8s activity. These studies demonstrate the complex interactions between cytokines and HIV replication/expression, involving both direct modulation of virus production from infected cells or indirect regulation via stimulation of host anti-viral activities.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00702-02 LIR

## PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Effect of HIV Infection on Gene Expression in CD4<sup>+</sup> T Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.M. Bende Staff Fellow LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID

A.L. Kinter Staff Fellow LIR, NIAID

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Immunoregulation

## SECTION

Immunopathogenesis Section

## INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

## TOTAL STAFF YEARS:

1

## PROFESSIONAL:

1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ © Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed a system to study the changes in gene expression in CD4<sup>+</sup> T lymphocytes that occur during human immunodeficiency virus (HIV) infection. Using mRNA differential display, we catalogued a number of genes which are differentially expressed between the human T-cell line A3.01, and its persistently HIV-infected daughter line, ACH-2. We have begun to exploit this technology to identify cellular genes whose expression is dysregulated upon acute infection of primary peripheral blood CD4<sup>+</sup> T cells with a primary viral isolate; we will also investigate the expression of candidate genes in lymph nodes of HIV-infected individuals. Ongoing work is aimed at verification/analysis of apparently affected genes to ascertain their role in HIV pathogenesis/immune dysregulation. These studies will enable us to dissect, at the molecular level, the mechanism of HIV-induced immune dysfunction.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00703-02 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Role of Dendritic Cells in the Pathogenesis of HIV Disease**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

PI: D. Weissman Senior Staff Fellow LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID  
Y. Li Visiting Associate LIR, NIAID  
T. Barker Guest Researcher LIR, NIAID  
K. Roche Study Coordinator LIR, NIAID

COOPERATING UNITS (if any)

George Washington Univ (J. Orenstein); Molecular Histolabs, Gaithersburg, MD (C. Fox);  
PRI, Frederick, MD (M. Baseler)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

2.75

PROFESSIONAL:

2

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects   ☒ (b) Human tissues   ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have continued to study the immunopathogenic role of dendritic cells in human immunodeficiency virus (HIV) infection and disease progression. The different populations of cells with dendritic morphology present in peripheral blood continue to be studied. This analysis has been extended to the observation that monocytes, when treated with GM-CSF and interleukin (IL)-4, develop a dendritic morphology. This population actually contains two distinct populations, one that expresses CD83, a marker of blood and lymphoid dendritic cells, and a second that expresses CD1a, a marker of Langerhans cells. A model has been developed that simulates the cellular interactions that occur in the microenvironment of lymphoid organs. This model is more physiologic than previous in vitro models in that the majority of viral replication occurs in the paracortical regions of the lymph node in activated, acutely infected CD4 positive T cells. Using this model, multiple activities produced by CD8 positive T cells have been identified. One is non-specific in that is found in CD8 positive T cells from uninfected individuals, while the other activity has only been identified in HIV-infected individuals and unlike the non-specific factor is gamma irradiation sensitive. It is likely that DC, as the most potent antigen presenting cell for primary immune responses, are involved in both the initiation and propagation of HIV disease. Epidemiological evidence from Africa suggests the HIV-disease has a more rapid course and a more efficient transmission compared to the United States which has been suggested to be due to immune activation. We have studied the effect of prior activation of T cells on the ability of DC to induce infection in CD4 positive T cells. Two weeks after tetanus immunization, DC can induce a more productive infection in CD4 positive T cells with 25 to 100 times less input virus. Thus, this enhancement of overall viral replication and ability to initiate an infection with much less virus during immune system activation demonstrates the peril of having an activated immune system upon exposure to HIV.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00721-01 LIR

## PERIOD COVERED

October 1, 1994 to September 30, 1995

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Follicular Dendritic Cells (FDC) in the Pathogenesis of HIV Disease.

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Y. Li Staff Fellow LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID

D. Weissman Senior Staff Fellow LIR, NIAID

J. Kehrl Senior Investigator LIR, NIAID

## COOPERATING UNITS (if any)

PRI, Frederick, MD (J. Adelsberger, M. Baseler)

## LAB/BRANCH

Laboratory of Immunoregulation

## SECTION

Immunopathogenesis Section

## INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

## TOTAL STAFF YEARS:

1.0

## PROFESSIONAL:

1.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ © Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lymphoid tissue is the major reservoir and site of replication of the human immunodeficiency virus (HIV). We have previously demonstrated that HIV virions are trapped extracellularly in the processes of the follicular dendritic cells (FDC) of the lymphoid tissue germinal centers. This trapping serves as a source of infectious virus to the CD4<sup>+</sup> T cells that migrate into the germinal centers to provide T-cell help in the induction of an HIV-specific immune response. Over time with HIV disease, the FDC network is destroyed and the lymph node architecture is disrupted. The mechanisms involved in the dissolution of the FDC network are unclear at present since infection of these cells with HIV is inefficient. The present study was aimed at characterizing the nature of the FDC as well as to establish cell lines for study of mechanisms of virus trapping and transfer of infection to CD4<sup>+</sup> T cells. Our studies have demonstrated that human FDCs are bone marrow-derived and share a common precursor with B lymphocytes. We also created an Epstein-Barr virus (EBV) transformed FDC-like cell line which can induce HIV expression in latently infected cell lines and bind to complement and antibody coated HIV particles through complement receptors similar to primary FDCs. These results will allow us to identify the FDC precursor cells and further to delineate the mechanisms responsible for the destruction of lymphoid tissue, CD4 T-cell killing, and viral expression and propagation in HIV-infected individuals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00701-02 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Therapy on HIV Viral Load in Lymph Nodes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	O. Cohen	Clinical associate	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	G. Pantaleo	Visiting scientist	LIR, NIAID
	C. Graziosi	Visiting scientist	LIR, NIAID
	M. Vaccarezza	Visiting fellow	LIR, NIAID
	S. Paolucci	Visiting fellow	LIR, NIAID

COOPERATING UNITS (if any)

George Wash U (J.Orenstein); Mol Histolabs, Gaithersburg, MD (C.Fox); PRI, Frederick, MD (G.Pavlakis); NCI (D.Schwartzentruber); NIAID (S.Schnittman); Palo Alto VA (M.Holodniy); U Oregon (M.Loveless); IPL, Carlsbad, CA (R.Moss)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously demonstrated that lymphoid organs are the major reservoir in vivo of human immunodeficiency virus type 1 (HIV-1). The effect of antiretroviral therapy on HIV burden and expression was evaluated by sampling peripheral blood (PB) and lymphoid tissue (LT) of patients at baseline and after 8 weeks of either remaining untreated, remaining on zidovudine (zdv), initiating zdv, or adding didanosine (ddI) to zdv. In individuals who did not undergo a change in therapy or who initiated zdv, patterns of histopathology, viral trapping, viral burden, and viral replication remained constant between week 0 and week 8. In patients who added ddI to ongoing zdv therapy, decreases in viral replication in LT were paralleled by decreases in plasma viremia, thus substantiating measurement of plasma viremia as a valid marker in assessing response to antiretroviral therapy.

The effects of an immunomodulatory agent, dinitrochlorobenzene (DNCB), in combination with Chinese herbs were evaluated by sampling PB and LN at baseline and after 6 months of initiating DNCB and herbs (DNCB treatment group) or herbs alone (control group). A significant decrease in CD4<sup>+</sup> T-cell counts was noted in both groups. Total CD8<sup>+</sup> T-cell counts remained unchanged; however, there was a significant increase in the percentage of CD8<sup>+</sup>CD38<sup>+</sup> cells in both groups. Viral burden and viral replication in PB and LN remained stable in both groups. Analysis of cytokine (interferon- $\gamma$ , TNF- $\alpha$ , IL-2, IL-4, IL-6, IL-10) expression from PBMC and LNMC revealed a TH<sub>1</sub>-like pattern at baseline which did not change at the 6 month time point in either group.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00675-03 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of HIV-Infected Long-term Non-Progressors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A.S. Fauci Chief LIR, NIAID

Others: G. Pantaleo Visiting Scientist LIR, NIAID  
S. Menzo Guest Researcher LIR, NIAID  
M. Vaccarezza Visiting Fellow LIR, NIAID  
C. Muro-Cacho Visiting Associate LIR, NIAID  
C. Graziosi Visiting Scientist LIR, NIAID

COOPERATING UNITS (if any)

Multicenter AIDS Cohort Study (MACS) Group; Duke Univ (D. Montefiori); George Washington Univ (J. Orenstein); Molecular Histolabs, Gaithersburg, MD (C. Fox);

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ © Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have studied a group (n=23) of HIV-infected individuals termed long-term non-progressors (LTNPs) whose disease has not progressed over periods ranging from 7 to 17 years. Levels of viral burden and virus replication were quite low in peripheral blood (PB) and lymph node (LN) mononuclear cells (MC) of LTNPs compared to progressors. Of interest was the fact that relatively high levels of plasma viremia were noted in the majority of LTNPs, comparable to levels in HIV-infected individuals whose disease had progressed. The degree of follicular hyperplasia and the total nodal and germinal center areas were significantly less in LTNPs. Of note, and in striking contrast to progressors, the lymph node architecture of the LTNPs were preserved despite several years of infection. Variable degrees of virus trapping was detected; several patients had little if any trapping of extracellular virions. Virus particles were virtually never detected in tissue or cell suspensions by electron microscopy. Only rarely were individual cells detected that were expressing HIV. The lack of virus trapping could in part explain the fact that although only very few cells were actively producing virus in LN, plasma viremia seemed not to be efficiently cleared leading to relatively high levels of plasma viremia. These data together with studies of lymphoid tissue in progressors suggests that disease progression is at least in part related to the deposition of virions on the follicular dendritic cell network of LN germinal centers, persistent activation of LN, active virus replication, and progressive destruction of lymphoid tissue. From an immunological standpoint, HIV-specific cytotoxicity against gag proteins was consistently observed in PBMC of LTNPs. Proliferative responses to a variety of stimuli (mitogens, alloantigens, and recall antigens) were preserved in PBMC of LTNPs. Characterization of humoral immune responses is currently underway. Intensive study of LTNPs should shed valuable insight into the pathogenic mechanisms of HIV disease and hopefully will provide important information for the development of therapeutic and vaccine strategies.



Cooperating United Continued:

FCRDC, Frederick, MD (Pavlakis); NCI (Schwartzentruber).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00361-13 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

International Studies of Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

PI: T.C. Quinn Senior Investigator LIR, NIAID

Other: A.S. Fauci Chief LIR, NIAID

COOPERATING UNITS (if any)

Johns Hopkins University (A. Rompalo, J. Zenilman, N. Halsey, G. Kelen, R. Bollinger, J. Bartlett)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The acquired immunodeficiency syndrome (AIDS) is a global pandemic with over 18 million human immunodeficiency virus (HIV)-infected individuals worldwide. A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic and immunologic features of HIV-1 and HIV-2 infections in developing countries and in the U.S. In Pune, India, we established a prospective cohort of over 3,000 high-risk individuals attending STD clinics, of whom 23% were seropositive for HIV-1. In a cohort of HIV-1 seronegative individuals followed over 15 months, the HIV incidence was 12.2% per year with rates as high as 28.6% for commercial sex workers. Recurrent genital ulcers and non-ulcerative STDs were independently associated with an 11 and 19-fold increased risk of HIV seroconversion, respectively. Phylogenetic analysis of viral strains isolated from these individuals demonstrate dissemination of both clade B and C strains with high-titer neutralizing antibody against clade B isolates. Additional studies among patients presenting to the Johns Hopkins Emergency Department demonstrated a rise in seroprevalence to 12.5% among 2,000 patients. A seroprevalence of 6.6% was documented by rapid HIV testing performed in the Emergency Department among patients of unknown serostatus. In a multi-center study comparing foscarnet and ganciclovir for the treatment of CMV retinitis, we demonstrated a significant decline in HIV-1 viral load in both treatment arms primarily due to their anti-viral effect on CMV replication. In perinatal studies in Zaire, Haiti, Malawi, and the U.S., we documented a 28% perinatal transmission rate. Utilizing HIV culture and PCR we estimated that 31% were infected in utero, 58% intrapartum, and 11% postnatally. In studies of HIV-1 and HTLV-1 co-infection in Brazil, we documented a higher incidence of myelopathy and peripheral neuropathy among dually infected individuals. In addition, T and B cell responses to pneumococcal and tetanus immunizations were markedly diminished among dually infected individuals compared to those infected with HIV-1 alone. Further studies are planned to further elucidate the immunologic modulations of HIV infection by co-infection with HTLV in these cohorts.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00390-12 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anti-Retroviral Therapy of Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.A. Polis Senior Investigator LIR, NIAID

Others: H.C. Lane Section Head LIR, NIAID  
R.T. Davey, Jr. Senior Investigator LIR, NIAID  
R.E. Walker Senior Investigator LIR, NIAID  
J. Falloon Senior Investigator LIR, NIAID

COOPERATING UNITS (if any)

CC/NIH (H. Masur, J.A. Kovacs, K. Spooner, S. Piscitelli); NEI/NIH (R. Nussenblatt, S. Whitcup); Chiron Corp (M. Urdea, J. Kolberg, S. Eastman, D. Chernoff); SAIC, Frederick, MD (N.P. Salzman, R. Dewar, M. Baseler, V. Natarajan)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

1

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

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☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An intensive effort was directed toward examining the relationships between changes in viral burden, changes in viral genotype and phenotype, and changes in immunologic function in patients with human immunodeficiency virus (HIV) infection receiving a variety of anti-retroviral agents with different mechanisms of action given alone or in combination. Interestingly, the majority of patients entering studies of new anti-retroviral agents did so with viral isolates containing mutations indicative of prior zidovudine exposure. The response to combination anti-retroviral therapy using 3 reverse transcriptase inhibitors simultaneously was clearly worse in those patients with a pre-existing mutation at the 215 codon. The patterns of viral resistance that emerged *in vivo* were quite distinct from those generated *in vitro*. Studies of the immunotoxin CD4-Pseudomonas exotoxin (CD4-PE) were conducted for the first time in humans. RNA-based methods were evaluated for the monitoring of viral burden. An infectious clone of HIV underwent an 8-base pair insertion in the region of the long terminal repeat. This mutant virus was replication competent and served as an excellent internal control for the measurement of particle-associated RNA in plasma. The branched-DNA (bDNA) technique was found capable of quantitatively measuring changes in viral burden in large numbers of samples to a significantly greater degree than existing techniques. The rate of CD4 count decline in a cohort of patients managed with combination anti-retroviral therapy was determined to be related to baseline levels of HIV RNA.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00635-04 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Trials for the Prevention and Treatment of HIV-Associated Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Falloon Senior Investigator LIR, NIAID

Others: H. C. Lane Section Head LIR, NIAID  
 R. Davey, Jr. Senior Investigator LIR, NIAID  
 M. Polis Senior Investigator LIR, NIAID  
 R. Walker Senior Investigator LIR, NIAID  
 M. Sneller Senior Investigator LIR, NIAID

COOPERATING UNITS (if any)

CC/NIH (H.Masur, J.Kovacs, K.Spooner, M.Piscitelli, C.Cartwright); SAIC, Frederick, MD  
 (M.Baseler, N.Salzman); CBER/FDA (J.Manischewitz); NEI/NIH (S.Whitcup, R.Nussenblatt)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ © Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

An intensive effort directed at improving the prevention and treatment of acquired immunodeficiency syndrome (AIDS)-associated opportunistic infections continues. A Phase I study of levofloxacin, an investigational quinolone, demonstrated that serum concentrations appropriate for the treatment of tuberculosis can be attained with safe, tolerable intermittent high-dose regimens. A study of pharmacokinetic drug interactions between stavudine, an anti-retroviral agent, and rifabutin or clarithromycin, drugs useful for the treatment or prevention of disease caused by Mycobacterium avium-intracellulare, has begun. An infrastructure for the collection of specimens from patients with tuberculosis has been established and assays for the detection and quantitation of M. tuberculosis in clinical specimens are being assessed. A study of the efficacy and pharmacokinetics of the investigational suspension formulation of atovaquone for the treatment of pneumocystis pneumonia has demonstrated a pharmacokinetic advantage of the suspension over the tablet formulation. A study of sulfasim, or CI-0694, a novel compound that can suppress and prevent antigen-specific antibody responses to sulfamethoxazole in animals, has just begun. This agent may prove useful in ameliorating the toxicity of trimethoprim-sulfamethoxazole and thus improve the treatment and prevention of pneumocystis pneumonia in some patients. A study of a ganciclovir-releasing intraocular implant with oral ganciclovir and an HIV-1 protease enzyme inhibitor is in the final stages of design. The assessment and follow-up of persons with idiopathic CD4+ T-lymphopenia (ICL) in order to investigate pathogenesis and natural history is continuing. Finally, an outreach effort for the enrollment of women and members of minority and medically under-served populations in clinical trials has begun which includes seeing patients in a local community health center as well as establishment of a city-wide AIDS clinical trials information center.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00636-04 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Approaches to the Therapy of HIV-1 Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

<u>PI:</u>	R. Walker	Senior Investigator	LIR, NIAID
<u>Others:</u>	H.C. Lane	Section Head	LIR, NIAID
	R.T. Davey, Jr.	Senior Investigator	LIR, NIAID
	J. Falloon	Senior Investigator	LIR, NIAID
	M. Polis	Senior Investigator	LIR, NIAID
	M. Sneller	Senior Investigator	LIR, NIAID
	A.S.Fauci	Chief	LIR, NIAID

COOPERATING UNITS (if any)

CC/NIH (H. Masur, J. Kovacs, K. Spooner, S. Piscatelli, S. Leitman, C. Carter);  
NCHGR/NIH (M. Blaese, L. Muul, R. Morgan); SAIC, Frederick MD (M. Baseler, V.

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

5.5

PROFESSIONAL:

2.5

OTHER:

3

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☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An intensive effort was directed toward studying the potential therapeutic aspects of immunologic interventions in patients with HIV infection. Ongoing studies evaluating the immunologic and antiviral effects of intermittent administration of interleukin (IL-2) and nucleoside analogues were continued. IL-2 therapy resulted in sustained increases in numbers of CD4 cells and decreased expression of activation markers on CD8 cells; the probability of manifesting these immunologic responses was shown to be directly associated with baseline CD4 count. Transient and consistent increases in viral load at the end of each infusion were revealed. Attempts to block cytokine induction by IL-2 with pentoxifylline in vivo failed to produce a clinical benefit. A randomized controlled trial comparing IL-2 plus nucleoside analogues to nucleosides alone was continued; a second randomized controlled multicenter trial was also continued comparing 3-, 4-, and 5-day infusions of IL-2 plus nucleosides to nucleosides alone. A dose escalation trial evaluating the safety and immunologic activity of subcutaneously administered IL-2 was expanded to include patients with less advanced disease. A study evaluating the immunologic and antiviral activity of intravenous IL-2 in combination with an inhibitor of HIV-1 protease was initiated. A study was begun to determine whether timing IL-2 therapy around laboratory correlates of immune activity produces greater and more durable responses than administering IL-2 on a fixed regimen. Studies evaluating the safety and activity of an anti-TNF antibody and a soluble TNF receptor were completed. A study comparing IL-2 administration alone to IL-2 combined with either anti-TNF antibody or thalidomide was begun. An anti-gp120 antibody derived from a recombinatorial library was identified and is being prepared for clinical development. A study evaluating the survival and distribution of adoptively transferred, genetically marked, syngeneic lymphocytes was undertaken; cells containing the marker gene continue to be detected in the peripheral blood of all 6 recipients from 28 to 48 weeks post-transfer. A gene therapy study was begun testing the safety and activity of repeated infusions of syngeneic CD8 cells engineered with a chimeric CD4 -zeta TCR receptor.



Cooperating Units (Continued)

Natarajan, R. Dewar, N. Salzman); MedImmune, Gaithersburg, MD (S. Koenig); Scripps Inst  
LaJolla, CA (D. Burton); Chiron Corp, Emeryville CA (G.Fyfe)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00634-04 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of the SCID-Hu Mouse Model of HIV-1 Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.C. Lane Section Chief LIR, NIAID

Others: M. Connors Medical Officer LIR, NIAID  
R.T. Davey, Jr. Senior Investigator LIR, NIAID

COOPERATING UNITS (if any)

SAIC, Frederick, MD (M. Baseler)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1

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- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The hu-HIV/PBL-SCID model was employed to explore the immunologic and virologic effects of different immune-based and antiviral therapies on the course of HIV-1 infection and disease. It was also used as a model to dissect the immunologic aspects of the human immune response to HIV, particularly in patients with stable disease. This model involves direct reconstitution of 8- to 12-week-old SCID mice with PBL from HIV-infected donors. Engraftment was achieved in 84% of mice as confirmed by both PCR detection of human cells and by detection of human antibody production and caused no diminution in the number of human mononuclear cells harvested by peritoneal lavage relative to control mice engrafted with uninfected cells. The human antibody produced by hu-HIV/PBL-SCID mice had broad reactivity against HIV. Virus was detected in 98% of mice by PCR or virus co-culture. Viremia was first detected by quantitative PCR on day 7 and persisted through day 17. Proviral DNA nucleotide sequences from peritoneal lavage cells recovered from hu-HIV/PBL-SCID mice on day 17 were not significantly changed from those derived from donor PBL at the time of injection. Reconstituted hu-HIV/PBL-SCID mice that were untreated sustained a 75% decrease in human CD4<sup>+</sup> T-lymphocytes recovered in peritoneal wash relative to control mice, whereas treatment with the nucleoside analogue FddA both significantly reduced CD4<sup>+</sup> cell depletion as well as diminished detectable virus. Boosting circulating human Ig antibody levels through injection of purified antibody derived from the donor's plasma with neutralizing activity did not affect the frequency of CD4<sup>+</sup> lymphocyte recovery or virus detection. The administration of a monoclonal antibody to human tumor necrosis factor-alpha at 10mg/kg resulted in only a modest increase in the CD4<sup>+</sup> T-lymphocyte survival. Higher doses were less effective. Studies were initiated to dissect the nature of the protective immune response in long term non-progressors and to examine the role of IL-12 as a therapeutic intervention.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00585-06 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenic Mechanisms in HIV and Other Retroviral Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

PI: D.I. Cohen Medical Officer LMSF, NIAID

Others: R. Lempicki Staff Fellow LIR, NIAID  
V. Kolesnitchenko Visiting Fellow LIR, NIAID  
R. Lowry Guest Worker LIR, NIAID  
H.C. Lane Section Head LIR, NIAID

COOPERATING UNITS (if any)

CBMB (L.Samelson); NCI (J.Ashwell); U Alabama, Birmingham (G.Shaw); Scripps (P.Russell), C.McGowan); Georgetown U (D.Hartmann, J.Cossman); U CA, San Diego (S.Hedrick, J.Newport)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Understanding the molecular mechanisms used by human immunodeficiency virus (HIV)-1 to kill CD4<sup>+</sup> T lymphocytes should contribute to finding new therapies capable of interrupting the progression to AIDS which follows HIV infection. Studies with peripheral blood T cells infected with primary clinical isolates of HIV-1 have shown that HIV-1 kills T cells by trapping the cells at a late point in the cell cycle (G2/M interface). These activated but arrested proliferating cells die when they are unable to complete the cell cycle and an abnormal form of programmed cell death (PCD) is initiated. The characteristics of this PCD, which include accumulation of proteins found only in cells about to enter mitosis, define the killing event as a pre-mitotic catastrophe. Working with cell lines expressing mutant forms of the cyclin dependent kinase p32cdc2 we were able to demonstrate that alterations in cdc2 kinase regulation triggers biochemical abnormalities that strongly resemble the biochemical abnormalities seen in the setting of HIV infection. Additionally, overexpression of bcl-2 was not seen in the setting of HIV infection and overexpression of bcl-2 was not able to block HIV-mediated PCD. We further demonstrated with a panel of metabolic inhibitors that HIV-mediated PCD substantially differed from the programmed death of CD4<sup>+</sup> T cells which occurs during normal T-cell development (negative selection). These observations have thereby increased the likelihood that clinically useful agents can be developed to selectively inhibit the HIV-killing process without initiating unacceptable toxicity in normal T cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00210-15 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulation of Human Lymphocyte Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

<u>PI:</u>	J.H. Kehrl	Senior Investigator	LIR, NIAID
<u>Others:</u>	A.S. Fauci	Chief	LIR, NIAID
	K. Druey	Medical Staff Fellow	LIR, NIAID
	V. Kang	IRTA	LIR, NIAID
	A. Riva	Visiting fellow	LIR, NIAID
	H. Zhang	IRTA	LIR, NIAID
	A. Himmelmann	Guest Researcher	LIR, NIAID

COOPERATING UNITS (if any)

Georgetown Med Ctr (P.Katz); Duke U, Durham, NC (T.Tedder); NCI, Frederick, MD (J. O'Shea); Harvard U, Boston, MA (J.Kryiakis) Washington U, St. Louis (K. Blumer)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

B Cell Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

7

PROFESSIONAL:

5

OTHER:

2

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- ☐ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Focusing on a novel protein termed RGP1 that is uniquely expressed in germinal center B lymphocytes, we discovered a new family of proteins (RGPs) and isolated six of the family members. These proteins are expressed in multiple tissues and based on complementation studies in yeast they likely regulate G protein linked signal transduction pathways. We isolated genomic clones spanning murine RGP1, mapped the RGP1 genomic locus, and created a targeting construct for homologous recombination in ES cells. Our studies also identified a sub-family of protein kinases homologous to yeast STE20. The prototype, GC kinase, localizes to the germinal center (GC) region in lymphoid tissue and activates the stress activated protein kinase (SAPK) pathway, a MAPK related pathway. We identified another member of this subfamily and are testing whether it also activates the SAPK pathway. Studies of the transcription factor BSAP identified a naturally occurring splice product that behaves as a dominant negative, discovered that the context of a BSAP cis-element alters its functional activity, determined that CD40 stimulation increased while protein kinase C activation decreased BSAP levels, developed an intracellular FACS assay for BSAP to measure its levels in defined populations of cells, and isolated the BSAP gene and identified its promoter. Studies of B lineage specific promoters identified a crucial PU.1 site in the Bruton's tyrosine kinase promoter; identified critical elements in the CD19 and CD22 promoters; determined that the co-activator protein BOB-1 is critical for the induction of CD20 and early B cell development. Studies of the B cell membrane protein CD22 revealed that cross linking of CD22 activated a potent signal transduction pathway and that the recruitment of SH-PTP1C likely limits signal transduction through the pathway. Finally, we localized bcl-x, but not bcl-2, in those B cells undergoing selection in the germinal center implying a role for bcl-x in the rescue of germinal center B cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00431-11 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Molecular Biologic Approach to Immune Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

<u>PI:</u>	U. Siebenlist	Section Chief	LIR, NIAID
<u>Others:</u>	T. Kanno	Visiting Associate	LIR, NIAID
	Y.-C. Lin	IRTA Fellow	LIR, NIAID
	H. Ellinger	Visiting Fellow	LIR, NIAID

COOPERATING UNITS (if any)

LP/NCI (K. Kelly)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immune Activation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

3

PROFESSIONAL:

3

OTHER:

0

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☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is focused on the elucidation of molecular mechanisms that operate during immune activation. In particular we have studied the genetic response of mitogenically activated T cells. Previously we have cloned many novel immediate-early induced genes of T cells. These genes encode for various transcription factors, including NF- $\kappa$ B, various cytokines, receptors and a number of intracellular signal-transducing components. We have now extended these studies to clone as yet undiscovered genes which are induced later during the cascade of events which ultimately lead to proliferation and full differentiation of the stimulated T cells. Activation of NF- $\kappa$ B is critical for immune function of stimulated T cells, since this factor regulates the expression of many immunomodulatory gene products; in addition, it regulates several viruses, including HIV. NF- $\kappa$ B also plays an important role during the virus transformation and activation of T cells by the HTLV I virus encoded Tax protein. We have discovered that Tax activates NF- $\kappa$ B by at least two mechanisms. The primary mechanism involves Tax-mediated degradation of the I $\kappa$ B- $\alpha$  inhibitory protein, the main cytoplasmic inhibitor of NF- $\kappa$ B. Tax causes the proteolytic degradation by inducing site-specific phosphorylation of the I $\kappa$ B- $\alpha$  inhibitor. We are studying the membrane-proximal events which occur during stimulation of immune cells with TNF- $\alpha$ , a potent activator of NF- $\kappa$ B. We have cloned as an immediate-early gene of T cells a member of a novel family of signal-transducing proteins which associate with the TNF receptor family. In addition, we are investigating the signaling path which leads from the T cell receptor to activation of NF- $\kappa$ B.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00723-01 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Activation of NF- $\kappa$ B, a Mediator of Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K. Brown Visiting Associate LIR, NIAID

Others: Y.-C. Lin IRTA Fellow LIR, NIAID  
 L. Baldi Visiting Fellow LIR, NIAID  
 U. Siebenlist Section Chief LIR, NIAID

COOPERATING UNITS (if any)

DCT/NCI (Weissman)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immune Activation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The research project concerns the mechanism by which the I $\kappa$ B- $\alpha$  inhibitor becomes inactivated during stimulation of immune cells which in turn leads to activation of the NF- $\kappa$ B transcription factor. NF- $\kappa$ B is critical for the induced expression of many genes whose encoded functions are required to counteract pathogens or stress. In addition, NF- $\kappa$ B regulates the expression of several viruses, including the human immunodeficiency virus (HIV). Therefore, blocking the activation of NF- $\kappa$ B could aid in the treatment of many inflammatory diseases and may also prevent the spread of HIV. An understanding of the mechanism(s) of activation of this transcription factor and the identification of the molecular components involved will provide potential targets for anti-inflammatory and anti-viral therapies. We have previously demonstrated that activation of the NF- $\kappa$ B transcription factor involves rapid phosphorylation and proteolytic degradation of its cytoplasmic inhibitor I $\kappa$ B- $\alpha$ . We discovered that the phosphorylation of this inhibitor in response to cellular stimulation is not sufficient to activate the transcription factor, but that induced proteolytic degradation is necessary. Specifically we found that calpain inhibitors will block activation of the transcription factor by blocking the proteasome-mediated degradation of I $\kappa$ B- $\alpha$ , while having no effect on its induced phosphorylation. We determined the precise amino acid sites at which the inhibitor becomes phosphorylated in response to signals, and we showed that phosphorylation of these sites is required for proteolytic degradation and thus for activation of NF- $\kappa$ B. These results will aid in the identification of the molecular component(s) which targets the phosphorylated inhibitor for proteasomal degradation and the kinase(s) which phosphorylates in response to appropriate stimuli.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00722-01 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunomodulatory Functions of the NF- $\kappa$ B/I $\kappa$ B Family

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Franzoso Visiting Associate LIR, NIAID

Others: U. Siebenlist Section Chief LIR, NIAID  
P. Biswas Visiting Fellow LIR, NIAID  
G. Poli Visiting Scientist LIR, NIAID  
A.S. Fauci Chief LIR, NIAID

COOPERATING UNITS (if any)

LMGD/NICHD (H. Westphal, P. Love); LVD/NIAID (J. Yewdell, J. Bennick); Oak Ridge National Laboratory (R. Woychik); FDA (W. Shores)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immune Activation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

1.25

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The NF- $\kappa$ B transcription factor family and the family of I $\kappa$ B proteins which regulate the function of these transcription factors are both critical for the defense of the organism since they control the expression of many immunomodulatory proteins as well as proteins which counteract stress. In addition, these proteins regulate the expression of HIV. The overall NF- $\kappa$ B and I $\kappa$ B activity in a given cell constitutes itself out of the functions of the individual family members which are expressed in that cell. An understanding of the unique functions of the various family members may offer highly specific and limited targets for immunomodulatory therapies. We have initiated a study to dissect the roles of specific individual members of these two families by generating so-called 'knock-out' mice which lack expression of either p52, a subunit of NF- $\kappa$ B, or Bcl-3, an I $\kappa$ B family member whose physiologic role is largely unknown. These mice will be challenged with viruses and pathogens to uncover specific roles of these proteins during immune responses. We are also generating transgenic mice which overexpress these and other family members. Since the p52 and Bcl-3 gene have been found as partners in recurrent chromosomal translocations in certain B- and T cell tumors, our studies will be essential to understand the role these proteins can play during tumorigenesis. Individual functions of the NF- $\kappa$ B complexes are also being investigated in the context of a given promoter. We discovered that some of the NF- $\kappa$ B transcription factors may exert their influence well beyond those genes which harbor known  $\kappa$ B binding sites in their regulatory regions; the NF- $\kappa$ B subunits p65 and c-Rel can interact with the serum response factor and thereby strongly and positively affect transcriptional activation through the SRE, the SRF binding element present in the regulatory regions of many signal-inducible genes. Finally, studies aimed at understanding the role of NF- $\kappa$ B during cellular infection with HIV uncovered a correlation between low infectivity and the presence of seprocidin proteases in monocyte/macrophages.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00358-13 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunopathogenesis of Chlamydia trachomatis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

PI: T.C. Quinn Senior Investigator LIR, NIAID

COOPERATING UNITS (if any)

Johns Hopkins University, (C. Gaydos, L. Bobo, R. Viscidi, S. West, J. Zenilman, A. Rompalo)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ © Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the U.S. with an annual incidence of 5 million cases. Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop improved diagnostic assays and to examine the pathogenesis of chlamydial infections. In screening 10,000 patients attending STD clinics, we have demonstrated that molecular amplification assays, including polymerase chain reaction (PCR) and ligase chain reaction (LCR), have markedly improved the sensitivity of detection of C. trachomatis. In screening urine samples by PCR or LCR, the prevalence of chlamydia infection was 14% among women attending STD clinics, 8% in family planning clinics, and 11% in adolescent high school clinics. In a new assay in which we co-amplify by PCR for detection of N. gonorrhoeae and C. trachomatis in male and female urine, infection rates were as high as 33.9% and 24.4% for chlamydia and gonorrhea among STD patients in the Philippines, Jamaica, and Malaysia. In transmission studies of 460 sexual partnerships, 52% were concordantly PCR positive. In contrast to culture results, the transmission efficiency was equal bidirectionally at 68%. In preliminary studies it appears that local production of TGF- $\beta$  in a predominant Th1 response to chlamydial infection is highly associated with the development of both an inflammatory and follicular trachoma, eventually leading to corneal scarring and blindness. Following our development of PCR for C. pneumoniae, we have identified C. pneumoniae in 8% of 700 patients with respiratory disease, with rates as high as 35% among immunocompromised patients. In vitro studies have demonstrated replication of C. pneumoniae in human pulmonary macrophages, human pulmonary artery endothelial cells, aortic artery endothelial cells, and aortic artery smooth muscle cells. After screening approximately 50 individuals with severe coronary atherosclerosis, we have demonstrated by culture, PCR, electron microscopy, and in situ hybridization, the presence of C. trachomatis in coronary atheromas in six patients. Additional studies are planned to further address the role of C. pneumoniae in respiratory disease and atherosclerotic heart disease.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00213-15 LIR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Immunopathogenic, Therapeutic Studies of Vasculitis and Other Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.C. Sneller Senior Investigator LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID

C. Langford Senior Staff Fellow LIR, NIAID

C. Talar-Williams Study Coordinator LIR, NIAID

COOPERATING UNITS (if any)

OSD/NIAID (C. Hallahan); LHD, NIAID (J.I. Gallin)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunologic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

3

PROFESSIONAL:

3

OTHER:

0

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

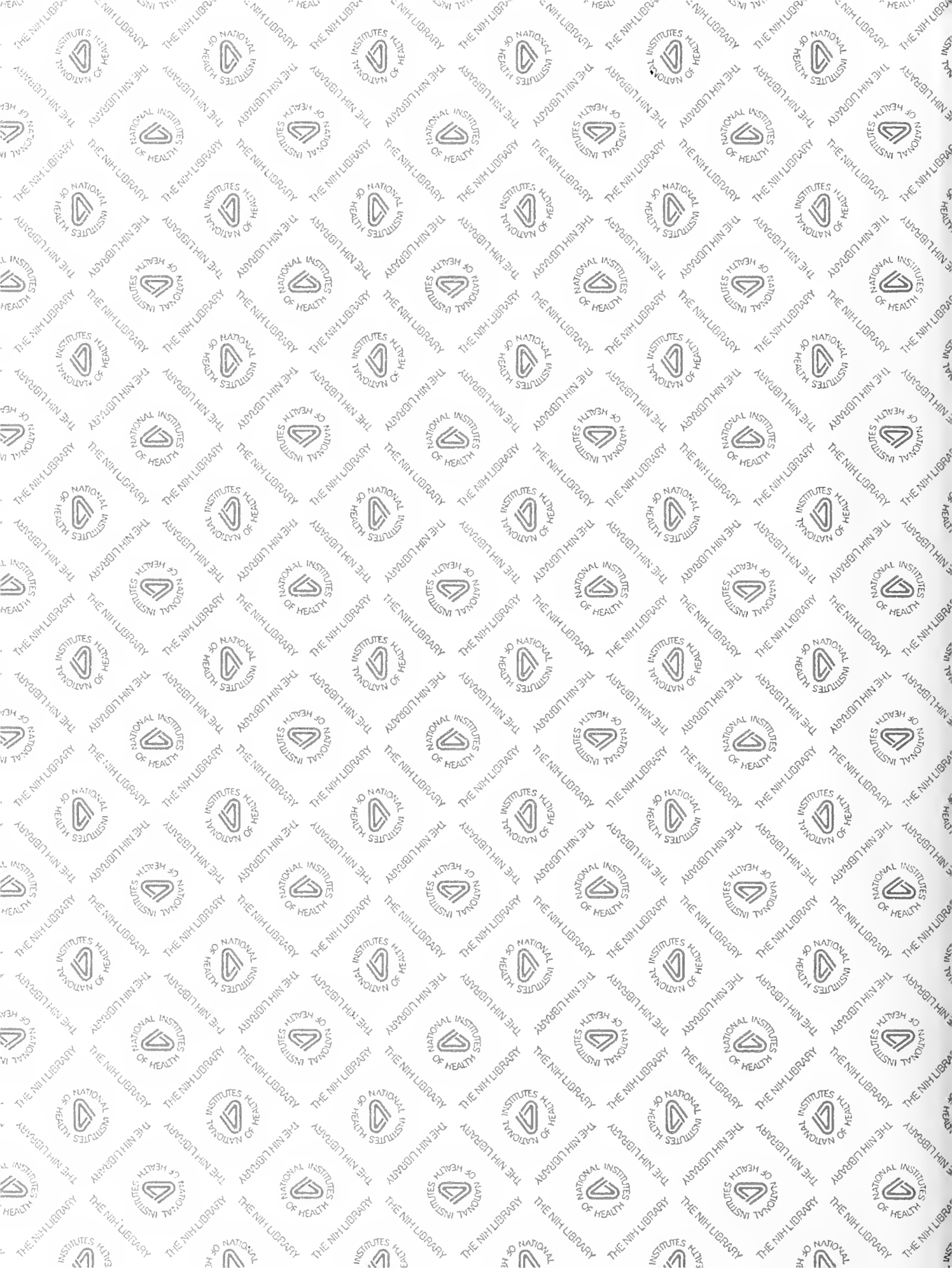
Following our previous therapeutic success with cyclophosphamide (CP) in the treatment of Wegener's granulomatosis (WG), we have gone on to evaluate the safety and efficacy of methotrexate (MTX) as an alternative therapy in this disease. Forty-two patients who did not have immediately life-threatening disease were studied. Glomerulonephritis was present in 21/42 (50%) of patients. The median follow-up was 33.7 months. Weekly administration of MTX and prednisone resulted in remission of disease in 34/42 patients (80%). The median time to remission was 4.2 months. Fifteen of the 34 patients achieving remission experienced a relapse of disease. The estimated median time to relapse for all patients achieving remission was 32 months. Ten patients who relapsed were treated with a second course of MTX plus prednisone. A second remission was induced in 8 out of 10 patients. Thus, MTX plus prednisone may be an acceptable alternative form of therapy for selected patients with WG.

In preliminary studies we have used skin blisters induced by suction as a method to study the in vivo inflammatory response of patients with WG. We have shown that patients with active WG produce 50- to 100-fold higher levels of TNF- $\alpha$  in blister fluid when compared with normal controls. Such high TNF- $\alpha$  levels are not seen in blister fluid from patients with a variety of other inflammatory and infectious diseases. Levels of IL-1 $\beta$  and IL-6 were also significantly elevated in blister fluid from patients with active WG. These findings have important therapeutic implications since specific inhibitors of TNF- $\alpha$  and other pro-inflammatory cytokines are currently available and may be effective in the treatment of WG and related vasculitic syndromes.

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